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THE UNIVERSITY OF ALBERTA

Chemical Studies of the Metabolites of *Lachnellula fuscosanguinea*

by

Jose Daniel Figueroa Villar

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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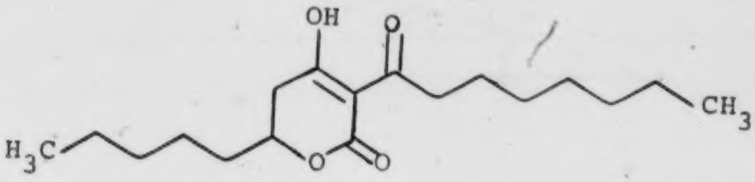
ABSTRACT

Lachnellula fuscosanguinea is known to be a strong antagonist in nature of the phytopathogenic fungi *Ceratosystis ulmi* (Dutch elm disease) and *Fomes pini*. The first chapter of this thesis deals with the isolation and structure elucidation of the metabolites produced by *L. fuscosanguinea*. Two metabolites responsible for the antifungal properties of *L. fuscosanguinea*, lachnelluloic acid (**6**) and lachnellulone (III, V) form a new family of active metabolites.



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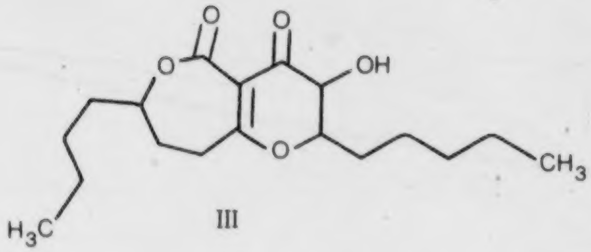

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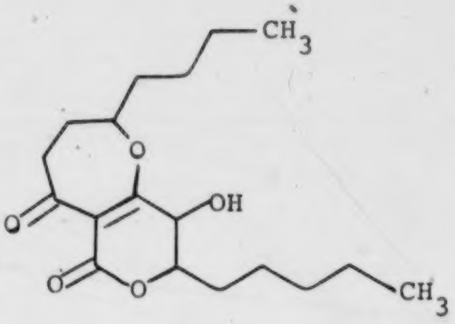

V

The second chapter of the thesis describes the total synthesis of lachnelluloic acid as final evidence to support

the proposed structure. The synthetic strategy has been designed to provide easy access to lachnelluloic acid and its structural analogues in order to assess some structure-activity relationships. The results of antibiotic bioassays of the natural products and their analogues are described in Chapter 2.

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TABLE OF CONTENTS

CHAPTER PAGE

Dedication....................iv

Abstract....................v

Acknowledgements....................vii

List of Tables....................ix

List of Figures....................x

Introduction....................1

Chapter 1....................4

- Results and Discussion....................4
- The Triglycerides....................8
- The Fatty Acids....................13
- Lachnelluloic Acid 16
- Lachnellulone....................34
- The nitrogen containing metabolite....................71
- The lachnelluloic acid analogue T-1....................72
- Fermentor extract....................73
- Experimental....................77

References....................110

Appendix NMR Spectra of the Metabolites....................112

Chapter 2....................122

- The total synthesis of lachnelluloic acid....................122
- Biological Activity....................163
- Experimental....................171

References....................200

LIST OF TABLES

TABLE PAGE

Chapter 1

I Fermentation studies on L. fuscosanguinea.........5

II Elution of the metabolites of L. fuscosanguinea from a flash column................................7

III Spectral data for lachnelluloic acid (**6**) and dihydrodehydroacetic acid (**8**)....................26

IV Gate decoupled spectrum of dihydrodehydroacetic acid...28

V Gate decoupled spectrum of D-labelled-dihydrodehydroacetic acid (**9**)...........................31

VI ^{13}C NMR chemical shift of sp^2-hydridized carbons in lachnellulone and lachnelluloic acid..38

VII Long range coupling of the Sp^2-hybridized carbons in lachnellulone.........................56

VIII Selective decoupling results for lachnellulone...58

IX Polarity gradient of elution of a flash column...82

X Usual elution of a 10 mm CLC disk...............83

XI Gate and selective decoupling of lachnellulone..102

Chapter 2

I Catalytic hydrogenation of dehydroacetic acid...156

II Bioactivity of crude extracts of Lachnellula fuscosanguinea against fungi....................165

III Antifungal activity of lachnellulone, lachnelluloic acid and analogues...................166

IV Kirby-Bauer test for lachnellulone, lachnelluloic acid and the synthetic analogues...............170

LIST OF FIGURES

FIGURE		PAGE
1	200 MHz ^1H NMR spectrum ($CDCl_3$) of the mixture of triglycerides	113
2	400 MHz ^1H NMR spectrum ($CDCl_3$) of lachnelluloic acid	114
3	200 MHz ^1H NMR spectrum ($CDCl_3$) of lachnellulone (III or V)	115
4	400 MHz ^1H NMR spectrum ($CDCl_3$) of isolachnellulone (VI or VII)	116
5	400 MHz ^1H NMR spectrum ($CDCl_3$) of lachnellulone-p-nitrophenyl hydrazone	117
6	200 MHz ^1H NMR spectrum ($CDCl_3$) of lachnellulone -p-nitrophenyl hydrazone diacetate	118
7	400 MHz ^1H NMR spectrum (Py-$\underline{d}_5$) of the nitrogen containing metabolite	119
8	400 MHz ^1H NMR spectrum (pyridine-$\underline{d}_5$) of compound T-1	120
9	400 MHz ^1H NMR spectrum (acetone-$\underline{d}_6$) of the yellow dye	121

INTRODUCTION

This thesis describes a part of the efforts made in these laboratories towards the understanding of the chemical basis of the interaction of phytopathogenic fungi with plants and other fungi.

Among the current problems being faced by mankind, the chemical pollution of the environment has become a threat to the future of this planet. An important cause of this problem is the use of artificial pesticides and fertilizers in agriculture. These artificial agents are an important tool in the production of food, shelter, energy and raw materials for our continually increasing population. Recently, the scientific community has recognized that a more rational approach to the solutions of the agricultural problems of pest control is the use of natural methods such as the employment of phytoalexins for weed control, and the use of pherormones to selectively destroy noxious insects. This approach has an advantage over the use of most artificial pesticides in that the natural agents are selective and biodegradable, and thus lead to less pollution. In addition, the use of natural agents has prompted the synthesis of structural analogues which have proven useful in human health and economy. Furthermore, the search for such biologically active compounds has led to the discovery of several drugs which are important in other areas of human and animal health. A substantial number of such compounds have been isolated from fungi.

One of the most important natural resources to man is wood. Wood is used as construction material and as a source of paper, chemicals, energy, combustibles and food. Perhaps the most serious problems in wood production are caused by fungi, since fungi are responsible for the destruction of standing timber, and in some cases of seedlings.

Some of the most phytopathogenic fungi are _Ceratosystis ulmi_, the causative agent of Dutch Elm disease, and _Fomes pini_, a wood rotting fungus.

C. ulmi is dispersed by beetles of the genus _Scolytus_, which introduce the spores of the fungus into the xylem of the trees.[1,2] The disease spreads rapidly over a tree once it has been infected, causing death in as little as a few weeks. _C. ulmi_ was first discovered in eastern Canada in 1944 and it has spread eastward and westward as far as Manitoba in the intervening years. This fungus was responsible for the destruction of 50% of the elm population of England in only two years, and for the disappearance of most of the elm population in eastern Canada and the U.S.A.

Fomes pini and other fungi of the genus _Fomes_ are responsible for the destruction of poplar and conifer in western Canada.

In 1979, Dr. A. Tsuneda from the Northern Forest Research Centre of The Canadian Forestry Service in Edmonton, discovered a strain of the fungus Lachnellula fuscosanguinea[3,4] growing on Pinus contorta in Blue River, British Columbia. This fungus, which is a member of the family Hyaloscyphaceae, was found to be an antagonist of C. ulmi and F. pini, both, in nature and in vitro.

Our interest in L. fuscosanguinea arises from the possible usefulness of the metabolites produced by this fungus in the control of forest and, possibly, human fungal diseases.

A literature search showed that no previous chemical work has been reported on L. fuscosanguinea or other fungi of the genus Lachnellula.

The purpose of this work is the isolation and structural elucidation of the metabolites responsible for the biological activity of L. fuscosanguinea. The problem involves the development of methods and conditions for the cultivation of the fungus, the design of assays for bioactivity, the separation and purification of the fungal metabolites, and the use of physical and chemical methods, including total synthesis, to assign the structure of the pure metabolites.

CHAPTER 1

RESULTS AND DISCUSSION

Lachnellula fuscosanguinea was cultivated in liquid nutrient media to produce large quantities of crude extracts. The liquid nutrient media used was potato dextrose broth (PDB), a medium simple to prepare and standardize, which allows reasonable growth of this fungus. The optimal temperature of fermentation was determined experimentally to be 17-20°C. Of the two common culture techniques, optimal still culture conditions were preferred over the use of a fermentor because these resemble more closely the natural conditions of growth for L. fuscosanguinea. It was also found that the total amount of crude metabolites produced in still culture is greater than that obtained by using a fermentor (see Table I). More importantly, no active metabolites were found in the crude extracts from the broth and mycelium obtained by culturing L. fuscosanguinea in the fermentor. The optimal time of growth, as determined by comparing the amount of metabolites produced with the time of growth, was found to be between 77 and 110 days. The results are summarized in Table I.

TABLE I. Fermentation studies on *L. fuscosanguinea*

Time of Growth (Days)	Still Culture Yield (mg/ℓ) Broth	Still Culture Yield (mg/ℓ) Myc	Still Culture Yield (mg/ℓ) Total	Fermentor Yield (mg/ℓ) Broth	Fermentor Yield (mg/ℓ) Myc	Fermentor Yield (mg/ℓ) Total
20	8.1	75.3	83.4	-	-	-
21	-	-	-	121.9	236.0	357.9
27	15.1	49.6	69.7	-	-	-
47	-	105.7	-	-	-	-
77	166.7	504.9	671.6	-	-	-
110	514.5	412.9	927.4	-	-	-

When the fermentor is used to cultivate *L. fuscosanguinea* the optimal growth period was found to be three weeks. After this period of time the culture begins to deteriorate. Yields of metabolites comparable to that obtained by optimal still culture conditions can are not be obtained. ~~possible~~. The fermentation mixture was separated into mycelium and broth by filtration, and the mycelium crude extracts were obtained by Soxhlet extraction of the air dried mycelium with ethyl acetate or dichloromethane. The broth was concentrated in vacuo (5 ℓ to 500 mL) and extracted with ethyl acetate in a continuous liquid-liquid extractor to afford the crude broth extract.

Two different chromatographic techniques were used for the preliminary separation of the crude mixture of metabolites; centrifugal liquid chromatography (CLC)[5] and flash chromatography.[6] CLC proved to be a good technique for the purification of the least polar components. However, this is a time consuming technique that needs simultaneous monitoring by analytical thin layer chromatography (TLC) and an ultraviolet (UV) detector.

The best preliminary separations of the crude extracts were accomplished by flash chromatography using a variation of the method of Still.[6] The amount of silica gel used was increased from 5-6 inches to 7-8 inches of column height, and the polarity of the solvent used for elution was gradually increased (see Experimental section). The elution order of the metabolites of L. fuscosanguinea from a flash column of the crude extract is shown in Table II.

TABLE II. Elution of the metabolites of *L. fuscosanguinea* from a flash column.

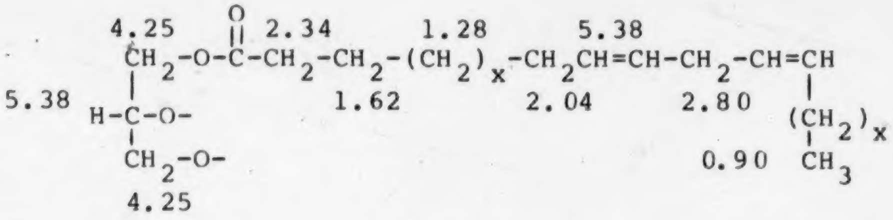
Fraction	Eluant	Composition
A-B	$CHCl_3$	mixture of triglycerides
C	$CHCl_3$	Lachnelluloic acid
D	$CHCl_3$	-
E	$CHCl_3$	Lachnellulone
F-H	$CHCl_3$-1% MeOH/$CHCl_3$	Fatty acids
I-K	1% MeOH/$CHCl_3$-5% MeOH/$CHCl_3$	minor products
L-M	10% MeOH/$CHCl_3$	nitrogen containing compound

The Triglycerides

A mixture of triglycerides was obtained directly from the crude extracts of L. fuscosanguinea using flash chromatography or CLC. This mixture of triglycerides was further purified by flash chromatography (Skellysolve B: ether) followed by column chromatography (CH_2Cl_2) to give a yellow oil which appears as a single component by TLC (Rf. 0.58 in $CHCl_3$).

The fourier transform infrared spectra (FTIR) of the triglyceride shows absorptions at 3000 cm^{-1} (vinyl protons), 1750 cm^{-1} (-COOR), 1660 and 1600 cm^{-1} (C=C).

The ^1H NMR spectra of this material shows a pattern characteristic of unsaturated triglycerides (Fig. 1):[7] δ 5.38 (9H, m), δ 4.25 (4H, 2 x dd). One of the nine protons at δ 5.38 corresponds to the C-2 hydrogen of the glyceride fragment. This proton was shown to be coupled to the protons at C-1 and C-3 of the triglyceride fragment (δ 4.25) by decoupling experiments. The other eight protons at δ 5.38 correspond to the vinylic protons of the acyl residues. This was shown by double irradiation experiments since saturation at the allylic hydrogens of the acyl residues (δ 2.04) produced decoupling at δ 5.38. A signal at δ 2.80 corresponds to doubly allylic hydrogens, implying that an unconjugated diene ($-CH=CH-CH_2-CH=CH_2-$) is present in the acyl chains. Finally, the signals at δ 2.34 and δ 1.62 correspond to the methylene protons α and β to the ester carbonyl, respectively. The final assignment of the ^1H NMR spectra is as follows:



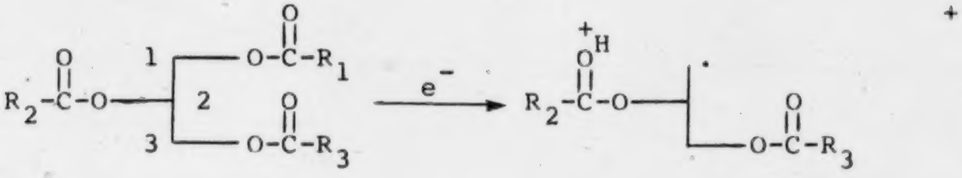
$$\begin{array}{l} CH_2\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_2\text{-}CH_2\text{-}(CH_2)_x\text{-}CH_2CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_x\text{-}CH_3 \\ | \\ H\text{-}C\text{-}O\text{-} \\ | \\ CH_2\text{-}O\text{-} \end{array}$$

The fact that the integration for the signal at δ 2.80 corresponds to only one hydrogen (see Experimental section) suggests that the component is a mixture of triglycerides. If this component were a single triglyceride, the intensity of the δ 2.80 signal should correspond to at least two hydrogens.

The high resolution electron impact mass spectrometry (HREIMS) results did not reveal a parent peak, but three important kinds of fragments are observed.[8]

1. Fragments containing four oxygens, which represent the loss of an acyloxy fragment from the triglyceride, are shown in Scheme 1.

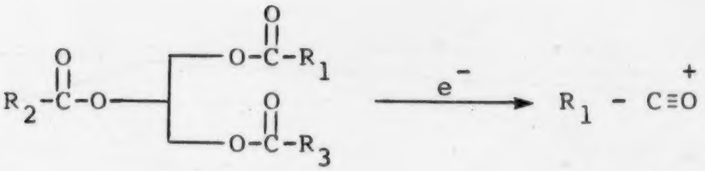
SCHEME 1:



Analysis of this type of fragmentation reveals the identity of the different acyl residues present in the triglyceride. Usually the least abundant tetraoxygenated fragment corresponds to the loss of the acyloxy residue at C-2. In the present case, three major tetraoxygenated fragments were detected: m/z 602 ($C_{39}H_{70}O_4$, 17%), 600 ($C_{39}H_{68}O_4$, 10%) and 577 ($C_{37}H_{69}O_4$, 75%). However, it is not possible to characterize the components because we are dealing with a mixture of triglycerides.

2. Fragments containing one oxygen atom, which are formed by cleavage of the ester -CO-O- bond, are shown in Scheme 2.

SCHEME 2:



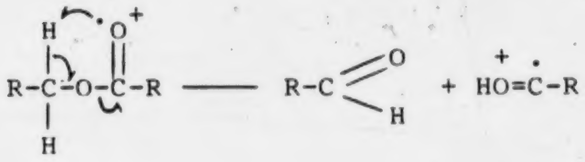
Three of those acyl ion fragments are detected (m/z 264 ($C_{18}H_{32}O$, 51%), 262 ($C_{18}H_{30}O$, 92%) and 239 ($C_{16}H_{31}O$, 19%)) which correspond to linoleic, linolenic and palmitic acid, respectively.

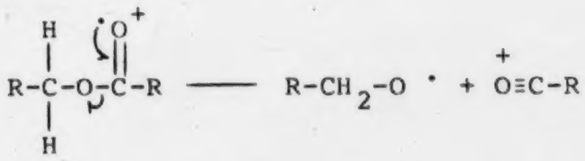
m/z 264 $H\overset{+}{O}=\overset{\cdot}{C}-(CH_2)_7-CH=CH-CH_2CH=CH-(CH_2)_4-CH_3$

m/z 262 $H\overset{+}{O}=\overset{\cdot}{C}-(CH_2)_7-(CH=CH-CH_2)_3-CH_3$

m/z 239 $\overset{+}{O}\equiv C-(CH_2)_{14}-CH_3$

Two fragmentation mechanisms are possible.

a) 

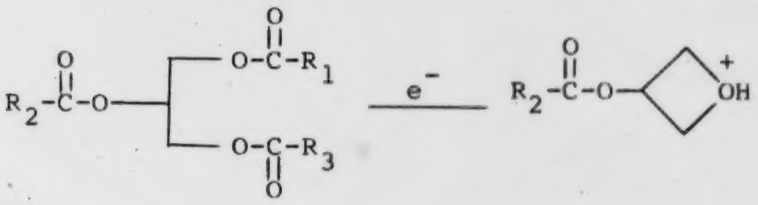
b) 

Mechanism (a) would be favored for the fragmentation of acyl residues at C-1 and C-3 of the triglyceride, since there are two carbinolic protons available for fragmentation and steric hindrance is minor. Mechanism (b) would be favored at C-2. If this hypothesis is true the palmitic acid residue is attached to C-2 of the triglycerides. This agrees with the fact that the least intense acyl ion usually corresponds to the acyl fragment at C-2 of a triglyceride.

In this case the weakest acyl ion corresponds to the palmitoyl fragment; m/z 239 ($C_{16}H_{31}O$, 19%). However, it is possible that for some other reason mechanism (a) is preferred for unsaturated acyl chains.

3. Fragments containing three oxygen atoms, which are formed by the loss of the acyl residues at C-1 and C-3 of the triglyceride, are shown in Scheme 3.

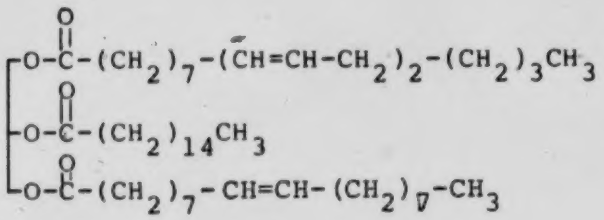
SCHEME 3:



This type of fragment is useful for determination of the acyl group at C-2 of a triglyceride. The formula of the corresponding acid is calculated by subtracting C_3H_5O from the molecular formula of the trioxygenated ion. In our case, three such fragments were detected: m/z 339 ($C_{21}H_{39}O_3$, 33%) which corresponds to an oleyl residue; 337 ($C_{21}H_{37}O_3$, 16%) which corresponds to a linoleyl residue, and 313 ($C_{19}H_{37}O_3$, 29%) corresponding to a palmitoyl residue. This result clearly indicates that the component is a mixture of at least three triglycerides.

Transesterification of the mixture of triglycerides with methanol and boron trifluoride etherate afforded a mixture of fatty acid methyl esters. The methyl ester mixture was analyzed by GC-Mass spectroscopy using an Apiezon L (10%) column.[9] Three major fatty acid methyl esters in a ratio of 2:1:2 were detected: methyl palminate (m/z 270), methyl linoleate (m/z 294), and methyl oleate (m/z 296).

In conclusion, it is evident that this oil is a mixture of triglycerides which differ by the relative position of the acyl residues and/or by their composition. For example, one of those triglycerides could have structure 1.



$$\begin{array}{l} -O-\overset{O}{\overset{\|}{C}}-(CH_2)_7-(CH=CH-CH_2)_2-(CH_2)_3CH_3 \\ -O-\overset{O}{\overset{\|}{C}}-(CH_2)_{14}CH_3 \\ -O-\overset{O}{\overset{\|}{C}}-(CH_2)_7-CH=CH-(CH_2)_7-CH_3 \end{array}$$

1

The Fatty Acids.

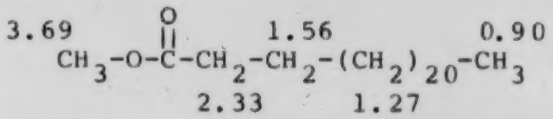
Fraction E from the CLC separation of the crude mycelium extract contains the major component of the fatty acid fraction. The major fatty acid was converted to its methyl ester by treatment with excess of ethereal diazomethane. Purification by column chromatography

(Skellysolve B: chloroform) gave the ester as a waxy solid (Rf. 0.56 Skellysolve B:chloroform: 1:1).

The FTIR of this product shows an ester carbonyl at 1740 cm^{-1}, and reveals the absence of carbon-carbon double bonds.

The HREIMS indicates a molecular weight of 382 ($C_{25}H_{50}O_2$) and the presence of only one unsaturation (the carbonyl). The fragmentation pattern of the ester follows the pattern of straight chain saturated methyl esters, with some diagnostic fragments: m/z 351 ($M-CH_3O$) and 74 (McLafferty fragment for methyl esters).

The ^1H NMR spectra of this ester show the presence of a methoxyl group at δ 3.69 (3H, s), a methylene group α to a carbonyl at δ 2.33 (2H, t), a methylene β to a carbonyl at δ 1.56 (2H, bt), a long methylene chain at δ 1.27 (40 H, b s) and a terminal methyl group at δ 0.90 (3H, vt). This compound was thus identified as methyl tetracosanate (**2**).



$$\overset{3.69}{\mathrm{CH_3}}\mathrm{-O-}\overset{\overset{\displaystyle \mathrm{O}}{\|}}{\mathrm{C}}\mathrm{-}\underset{2.33}{\mathrm{CH_2}}\mathrm{-}\overset{1.56}{\mathrm{CH_2}}\mathrm{-}\underset{1.27}{\mathrm{(CH_2)_{20}}}\mathrm{-}\overset{0.90}{\mathrm{CH_3}}$$

2

Other fatty acids that have been detected in the extracts of *L. fuscosanguinea* are stearic acid (**3**), linoleic acid (**4**) and palmitic acid (**5**) (fractions F to H from the flash column of the crude extract).

$CH_3-(CH_2)_{16}-CO_2H$

(3)

$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-CO_2H$

(4)

$CH_3-(CH_2)_{14}-CO_2H$

(5)

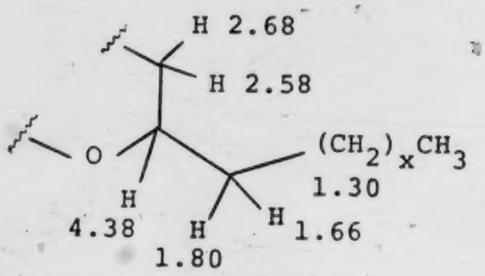
Lachnelluloic Acid (**6**).

Lachnelluloic acid was isolated from the crude mycelium extracts of L. fuscosanguinea in the following way. Flash chromatography of the mycelial extract gave several fractions (Table I). Purification of fraction C by column chromatography over silica gel (Skellysolve B-chloroform 1:1) gave a single compound (TLC). This colorless, needlelike crystalline compound has a low melting point (39-40°C) and is soluble in most organic solvents. Lachnelluloic acid may, at times, amount to as much as 13% of the crude mycelium extract from L. fuscosanguinea, but at other times it is present in very small quantities. The colorless crystals of lachnelluloic acid slowly become yellow and finally orange when left exposed to the atmosphere, however, repurification by flash chromatography is possible without appreciable loss of material.

The FTIR of lachnelluloic acid does not show the presence of a hydroxyl group.[10] In the carbonyl region of the spectrum, a medium intensity signal at 1708 cm^{-1} and a strong band at 1695 cm^{-1} (C=C-C=O) are the only absorptions. A broad signal at 1555 cm^{-1} suggests the presence on an enolized β-dicarbonyl moiety (chelated carbonyl).[11,12] The presence of the enol was confirmed by a positive ferric chloride test.

The HREIMS and chemical ionization mass spectrometry (CIMS) results indicate a molecular weight of 310 ($C_{18}H_{30}O_4$), and the presence of four unsaturation equivalents in lachnelluloic acid. The fragmentation pattern of the molecule shows the presence of a highly oxygenated molecular nucleus (m/z 239 ($C_{13}H_{19}O_4$, 100%), 226 ($C_{12}H_{18}O_4$, 49%), 155 ($C_7H_7O_4$, 25%), 141 ($C_6H_5O_4$, 18%) and 129 ($C_5H_5O_4$, 17%)), which contains all four oxygen atoms present in lachnelluloic acid.

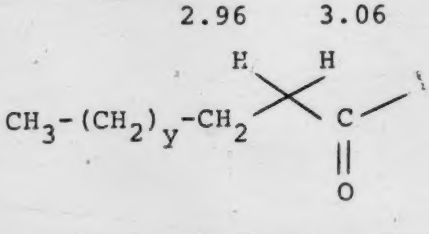
The ^1H NMR spectra of lachnelluloic acid (Fig. 2) are very informative. All 30 hydrogen atoms are detected by ^1H NMR as two independent spin systems and an isolated low field singlet. When the signal at δ 4.38 (1H, dddd, J = 4, 4, 7, 11 Hz) is irradiated, multiplicity changes are observed at δ 2.68 (1H, dd, J = 11, 17, Hz), δ 2.58 (1H, dd, J = 4, 17 Hz), δ 1.80 (1H, dddd, J = 5, 7, 10, 12 Hz) and δ 1.66 (3H, m). The signals at δ 2.68 and 2.58 both collapse to a doublet (J = 17 Hz) and further irradiation experiments show that the protons giving rise to these signals are geminal. The chemical shift of these methylene protons suggests that they are vicinal to a carbonyl or a carbon-carbon double bond. The signals at δ 1.80 and δ 1.66 also form part of a geminal system (J gem = 13 Hz), and since saturation of these signals induces decoupling in the absorption region of saturated methylene (δ 1.3), fragment A becomes evident.



A

The other spin system present in lachnelluloic acid shows two geminal protons vicinal to a carbonyl at δ 3.06 (ddd, J = 6.5, 8.5, 15 Hz) and δ 2.96 (ddd, J = 6.5, 8.5, 15 Hz). Irradiation of these methylene protons produces a change in the coupling pattern of the signal at δ 1.66, the integration of which corresponds to three hydrogens: one belonging to the spin system of fragment **A**, the other two belonging to a geminal pair of methylene protons which are part of a long aliphatic chain.

The presence of a second aliphatic chain in lachnelluloic acid is evident in the ^{1}H NMR spectra since two virtually coupled methyl groups (δ 0.92 and 0.90) are present. Thus fragment **B** is indicated.



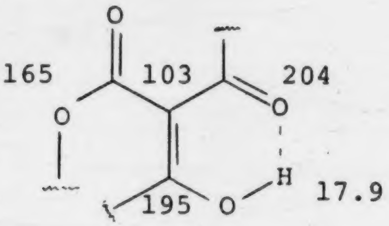
B

The ^{13}C NMR spectra of lachnelluloic acid show the presence of four sp^2 hybridized carbons at δ 209 (s), δ 195 (s), δ 164 (s) and δ 103 (s); an oxygen bearing carbon at δ 74 (d); eleven methylenes (all triplets) and two methyl groups.

The number of hydrogen atoms attached to carbon in a molecule can be counted from the ^{13}C signal multiplicity. In lachnelluloic acid, 18 carbons can be accounted for, which by their multiplicity indicate the presence of 29 C-H bonds (or 29 hydrogen atoms) in the molecule. This indicates that the 30th hydrogen atom is attached to oxygen, and therefore a hydroxyl group is present. The hydroxyl group does not appear in the FTIR spectrum of lachnelluloic acid, but it can be detected in the ^1H NMR spectra as a sharp one proton singlet at δ 17.9 which exchanges with D_2O. This type of hydroxyl group resembles those of the enolic form of β-diketones.[13]

Since only two isolated spin systems can be observed in the ^1H NMR spectra of lachnelluloic acid, the four sp^2 hybridized carbons seen in its ^{13}C NMR must join fragments A and B. The carbon at δ 204 is part of a ketonic carbonyl while the carbon at δ 103 is the electron rich carbon of a highly polarized carbon-carbon double bond.[14] Of the two sp^2 hybridized carbons left, the one at δ 164 is part of the carbonyl of an ester or a lactone and the one at δ 195 must be the electron deficient end of the polarized double bond.

There is only one way to arrange four sp^2 hybridized carbon atoms to account for these shifts and at the same time account for the presence of the enol of a β-dicarbonyl system. This arrangement is shown in fragment **C**.

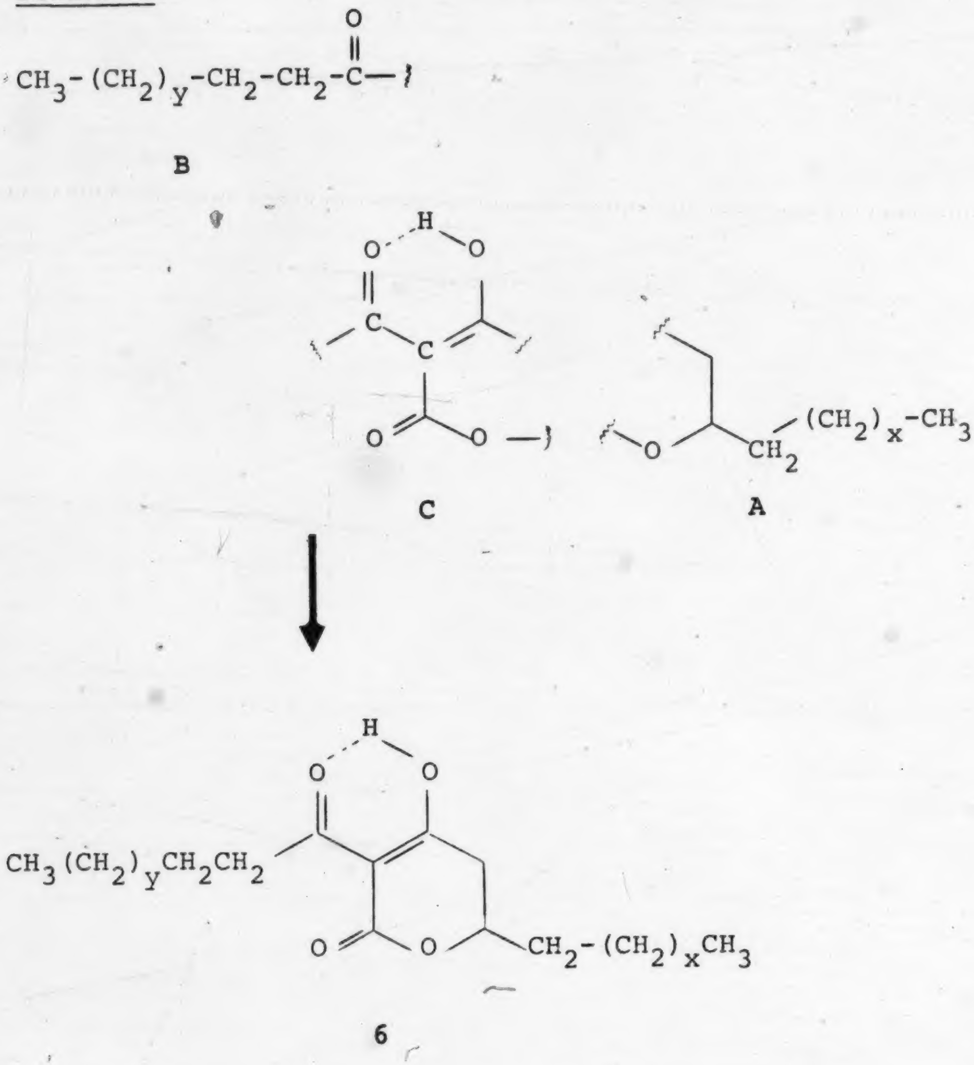


C

Since lachnelluloic acid contains only four oxygen atoms, which are all present in the partial structure **C**, the oxygen atom shown in partial structure **A** must be the same as the ether oxygen of the ester in **C**. Furthermore, the carbonyl in fragment **B** must be the same as that ketone carbonyl in **C**. Thus lachnelluloic acid must have structure **6**, as shown in Scheme 4.

Structure **6** only accounts for four methylene carbons. Since lachnelluloic acid possesses eleven methylenes the remaining seven methylene groups form part of the fragments **A** and **B**. The arrangement of methylenes can be determined from the equation $x + y = 7$. In order to account for the virtual coupling of the aliphatic methyls of lachnelluloic acid the number of equivalent methylenes in each chain must

SCHEME 4:

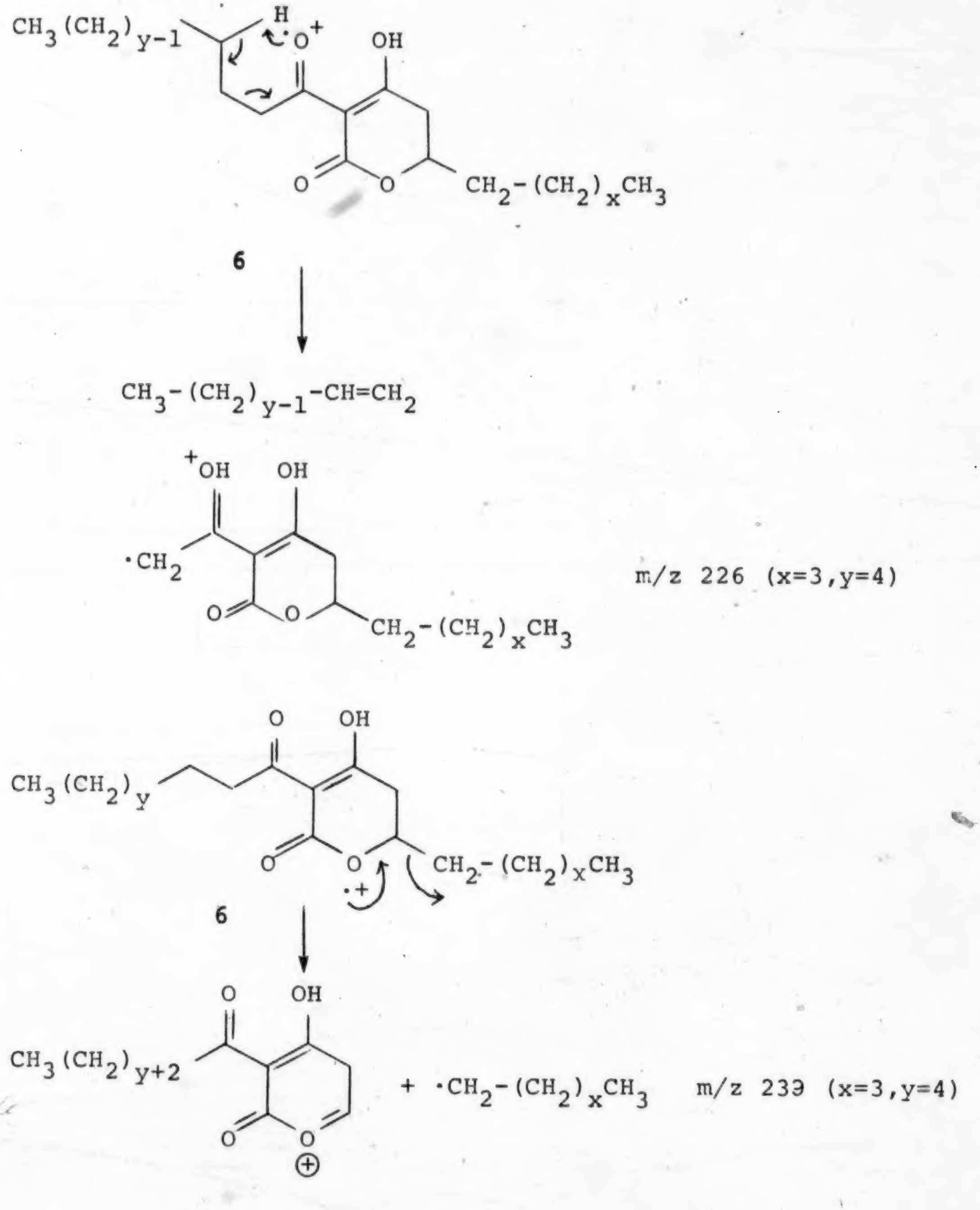

CH3-(CH2)y-CH2-CH2-C(=O)—
B
O··H··O
C
O
O
A
(CH2)x-CH3
CH2
C
↓
CH3(CH2)yCH2CH2
H
O
O
O
CH2-(CH2)xCH3
6

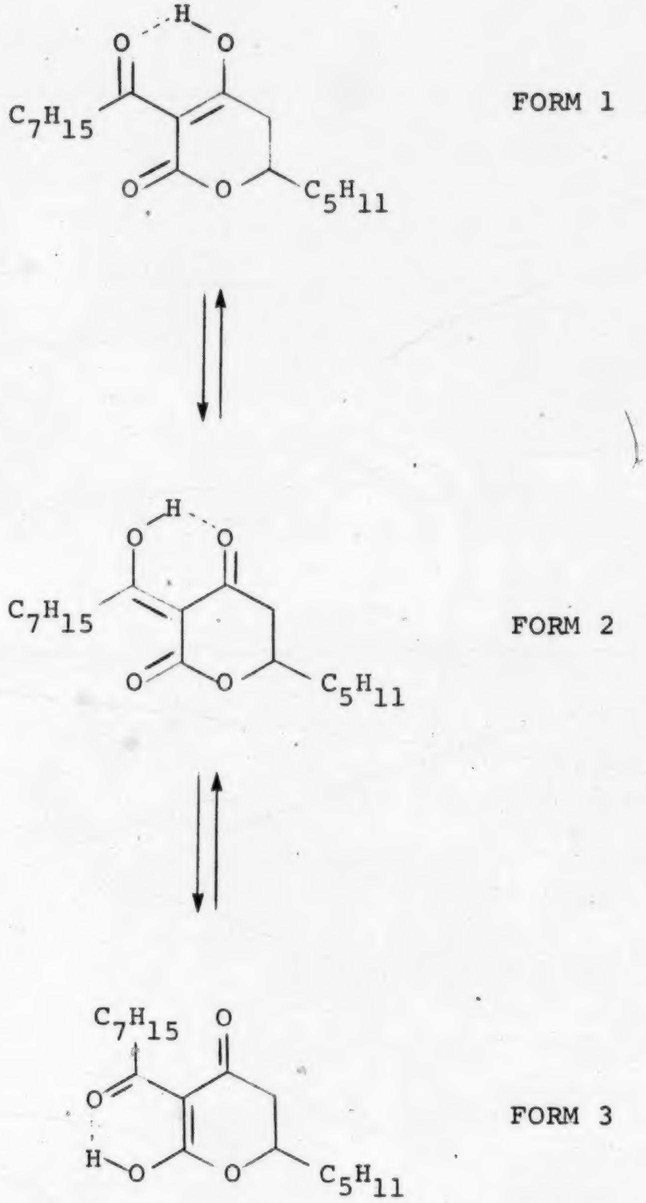
be 2 or more, therefore the only possible combinations of x and y are 2 and 5 or 3 and 4.

The most intense peak in the HREIMS of lachnelluloic acid, m/z 239 ($C_{13}H_{19}O_4$), corresponds to a loss of C_5H_{11} from the molecular ion. This fragmentation indicates that one of the aliphatic chains is at least 5 carbons long. Another abundant fragment corresponds to a loss of C_6H_{12}, m/z 226 ($C_{12}H_{19}O_4$), thus one of the side chains must contain at least 6 carbon atoms. These fragmentations can be explained in structure **6** by a McLafferty fragmentation and an ether type fragmentation when x =3 and y = 4, as shown in Scheme 5. Conclusively, lachnelluloic acid must possess structure **6**, in any one of the three enolic forms shown, or in equilibrium.

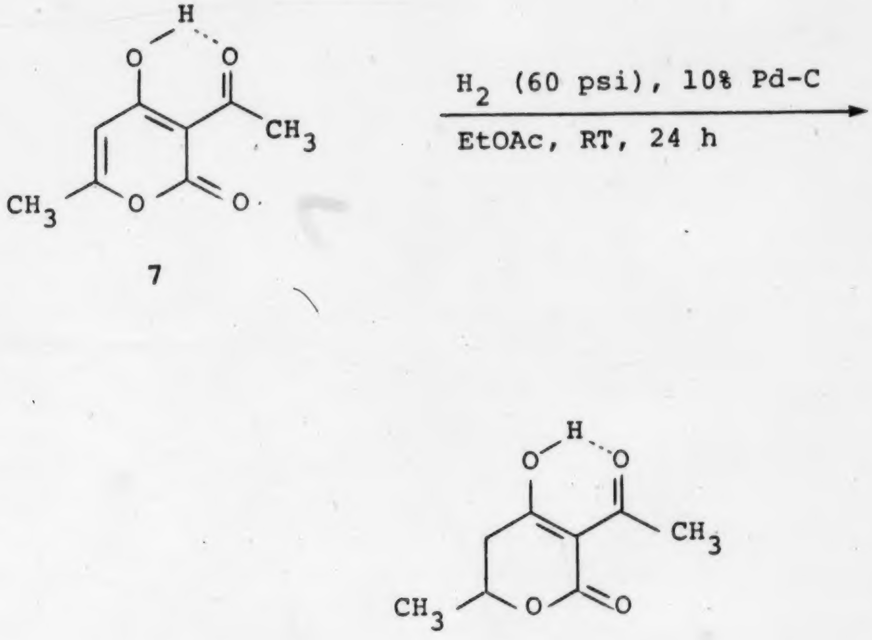
In order to obtain a model compound for lachnelluloic acid, dehydroacetic acid (**7**) was hydrogenated in ethyl acetate using 10% Pd-C as catalyst.

SCHEME 5:


CH3(CH2)y-1
H
O+
OH
O
O
CH2-(CH2)xCH3
6
CH3-(CH2)y-1-CH=CH2
+OH
OH
·CH2
O
O
CH2-(CH2)xCH3
m/z 226 (x=3,y=4)
O
OH
CH3(CH2)y
O
O
·+
CH2-(CH2)xCH3
6
O
OH
CH3(CH2)y+2
O
O
+ ·CH2-(CH2)xCH3
m/z 239 (x=3,y=4)


FORM 1
FORM 2
FORM 3
C7H15
C5H11
H
O

6



7

8 (~ 100%)

The product of hydrogenation was purified by flash chromatography to afford in quantitative yield (based on recovered starting material) the known dihydrodehydroacetic acid (**8**)[15] as a white crystalline compound (mp 88-89°C).

The spectral characteristics of lachnelluloic acid (**6**) and the model compound (**8**) are compared in Table III.

Table III. Spectral data for lachnelluloic acid (**6**) and dihydrodehydroacetic acid (**8**).

Type of Spectra	Lachnelluloic Acid	Dihydrodehydroacetic Acid
^1H NMR ($CDCl_3$)	δ 17.9, 4.38, 3.06, 2.96 2.68, 2.58, 1.80	δ17.9, 4.65, 2.70, 2.68 and 1.50
^{13}C NMR ($CDCl_3$)	δ 204, 195, 164, 103 74, 38	δ201, 195, 164, 103, 70 and 39
FTIR ($CHCl_3$)	ν_{max} 1708, 1695 and 1555 cm^{-1}	ν_{max} 1715 and 1560 cm^{-1}
UV (MeOH)	λ_{max} 218, 274 nm	λ_{max} 219, 274 nm
UV(+NaOH)	λ_{max} 250 nm	λ_{max} 248 nm
UV (+HCl)	λ_{max} 218, 274 nm	λ_{max} 214, 274 nm

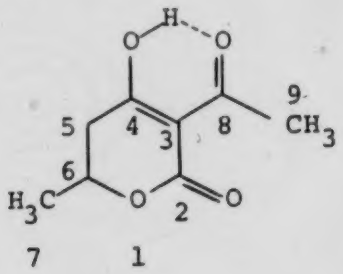
From Table II it can be seen that the two compounds are closely related. The similarity of the ultraviolet spectra, which clearly indicates that both compounds contain the same chromophore, i.e., the same arrangement of sp^2 hybridized carbons is especially important.

It remains to determine the enolic form of lachnelluloic acid. This problem was resolved by the analysis of a totally coupled ^{13}C NMR spectrum of the model compound, dihydrodehydroacetic acid (**8**). The determination of a coupled ^{13}C NMR spectrum was accomplished using a pulse sequence that allows the generation of the nuclear Overhauser enhancement (NOE) in all the carbons, without eliminating the carbon-hydrogen coupling. This technique is known as gated decoupling;[16] the broad band decoupling mode (BB) is turned on until the NOE is generated in every carbon, then the decoupler is turned off, and after a short delay time the free induction decay (FID) of the sample is recorded. By means of this technique long range carbon-hydrogen coupling constants can be detected and measured. When data manipulation processes such as zero filling[17] and resolution enhancement[18] are used, a remarkable improvement in the clarity of the coupling patterns in the gated spectrum is usually observed.

The results of this experiment for dihydrodehydroacetic acid (**8**) are shown in Table IV.

Table IV. Gated decoupled spectrum of dihydrodehydroacetic acid.

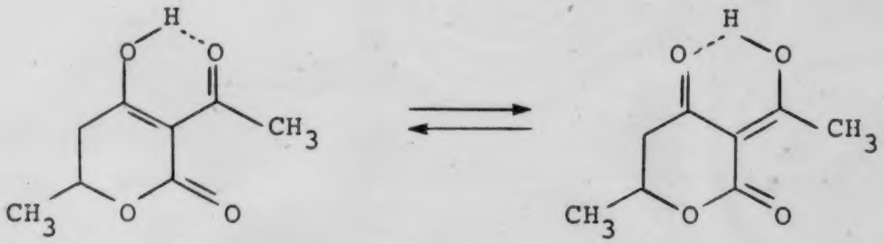
Carbon	Chemical Shift δ (ppm)	Multiplicity	Coupling Constant J (Hz)
8	201	qd	6.5, 2.5
4	195	m	-
2	164	bs	-
3	103	m	-
6	70	dq	146.0, 3.5
5	39	tq	120.5, 5.0
9	26	qbd	129.5, 1.5
7	20	qq	117.5, 3.0



8

In this spectrum $^1J_{C-H}$, $^2J_{C-H}$ and $^3J_{C-H}$ can usually be determined, although in some cases $^2J_{C-H}$ and $^3J_{C-H}$ are not observed. For example, the signal at δ 70 should appear as a doublet of quartets of triplets, however, the coupling with the neighboring methylene is not observed.[19] It is important to note the multiplicity of the sp^2 hybridized carbons which shows that coupling through a hydrogen bond is possible.[20] The signal at δ 201 (C-8) appears as a doublet of quartets rather than as a simple quartet.

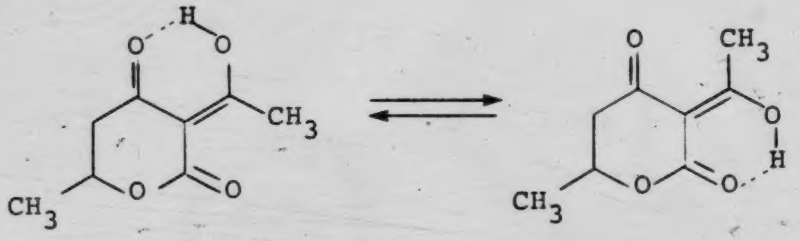
Enolization of the lactone carbonyl has not occurred since the signal at δ 165 (C-2) appears as a broad singlet. This leaves the possibility of enolization of the carbonyl in the ring (C-4) or of the carbonyl in the side chain (C-8). The fact that only one enolic form is detected indicates that either equilibration is rapidly taking place between forms 1 and 2 (see page 24); or that one of the two enolic forms is favored over the other



FORM 1

FORM 2

However, enolization at the side chain carbonyl can lead to two possible enols, as shown below.

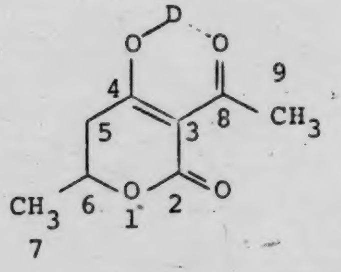


FORM 2a FORM 2b

Again, a single enol would be detected by NMR if the equilibration between form **2a** and form **2b** is fast for the NMR time scale. It may be possible that one enolic form is thermodynamically favored over the other, probably form **2a** because it contains a hydrogen bond with a more polarized ketone carbonyl. The gated decoupled ^{13}C NMR spectrum of monodeuterated dihydrodehydroacetic acid, obtained by exchange with CD_3OD, was determined to discriminate between these possibilities. The results are summarized in Table V.

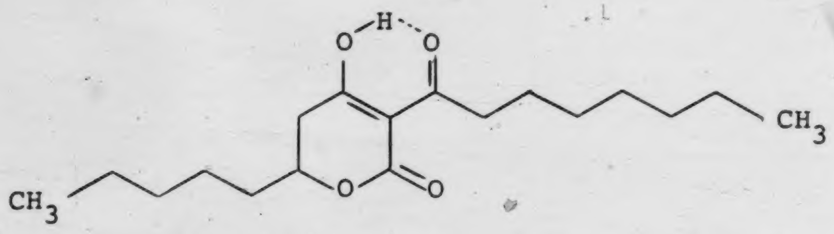
Table V Gated decoupled spectrum of D-labelled-dihydro-dehydroacetic acid (**9**).

Carbon	Chemical Shift (δ)	Multiplicity	Coupling Constant (Hz)
8	201	q	6.5
4	194	m	-
2	164	bs	-
3	103	m	-
6	70	ddm	147.5, 3.5
5	39	tbq	130.0, 4.5
9	26	q	130.0
7	20	qq	127.5, 2.0



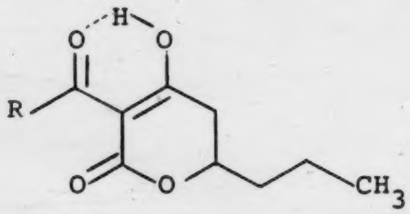
9

The lactone carbonyl is clearly not enolized, as indicated by the fact that the exchange of the enolic proton of **8** for deuterium does not affect the multiplicity or the chemical shift of C-2. For the same reasons, it can be concluded that form **2b** is not present in solution as part of an equilibrium or as the most stable form. This leaves as the only possibilities form **2a**, form **1** or an equilibrium between them. It is known that in six-membered ring systems endocyclic double bonds are usually more stable than exocyclic double bonds.[21] Therefore dihydrodehydroacetic acid and lachnelluloic acid should exist in enolic form **1** or in equilibrium between forms **1** and **2a**.



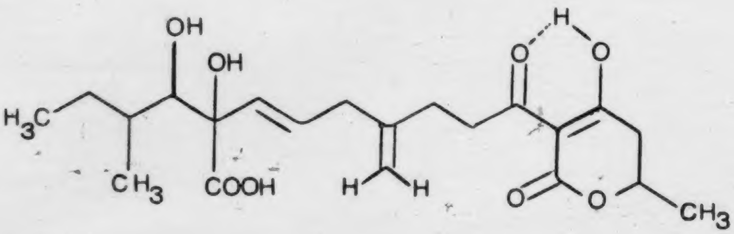
Lachnelluloic Acid

Miyakado and co-workers recently reported the structure of the antifungal podoblastins,[22] isolated from Podophyllum peltatum, providing us with naturally occurring structural analogues of lachnelluloic acid. Podoblastins A (**10**), B (**11**) and C (**12**) differ from lachnelluloic acid only in their side chains. The reported carbon and proton assignments in the ^{13}C NMR and ^{1}H NMR of the podoblastins are in agreement with our assignments for lachnelluloic acid.



10 (R = $CH_3-(CH_2)_{10}-$)
11 (R = $CH_2=CH-(CH_2)_8-$)
12 (R = $CH_3-(CH_2)_{12}-$)

Another natural product containing the same dihydropyrone moiety is the fungal metabolite alternaric acid.[23]



Alternaric acid

Lachnellulone.

Recrystallization, from Skellysolve B, of fraction E from the flash chromatography separation of the crude mycelium extract of L. fuscosanguinea produced a white crystalline material (mp 126-127°C, Rf 0.44 in $CHCl_3$). This compound, which was named lachnellulone, accounts for as much as 1.3% of the crude mycelium extract, although it is generally found in smaller quantities or not at all. Lachnellulone can also be isolated from the broth extract of L. fuscosanguinea, but in small amounts. This optically active metabolite ($[\alpha]_D^{25}$ + 48.4 (c 10, CH_3OH) exhibits a strong antifungal activity against Ceratosystis ulmi. This, together with its structural complexity, make lachnellulone the most interesting metabolite isolated from L. fuscosanguinea.

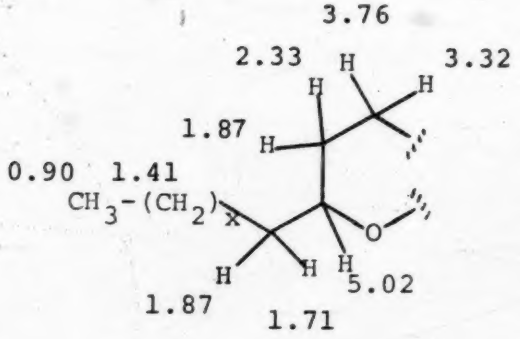
The FTIR of lachnellulone shows the presence of a hydroxyl group (3400 cm^{-1}), two conjugated carbonyls (1708 and 1670 cm^{-1}) and a band characteristic of the enol form of β-dicarbonyl compounds (1560 cm^{-1}, broad).

The UV spectrum of lachnellulone (λ_{max} 218, 275 nm) is strikingly similar to that one of lachnelluloic acid (λ_{max} 218, 274 nm). This resemblance is extended to the UV spectrum, when it is recorded in the presence of a trace of base. The maxima observed are as follows (λ_{max}

(lachnellulone) 255 nm, λ_{max} (lachnelluloic acid) 250 nm). This hypsochromic shift is reversed by acidification. These results suggest that lachnellulone and lachnelluloic acid contain the same highly oxygenated chromophoric system.

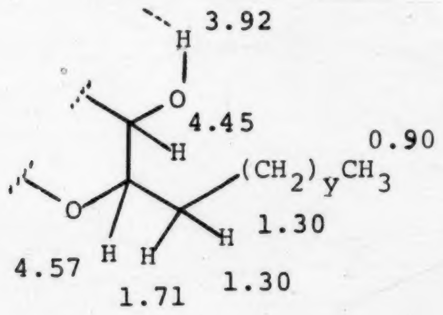
The HREIMS of lachnellulone shows a molecular weight of 324 ($C_{18}H_{28}O_5$), which was confirmed by chemical ionization mass spectrometry (CIMS). The most prominent fragments that are detected are m/z 308 ($[M-O]^+$), 239 ($[M-C_5H_9O]^+$), 226 ($[M-C_6H_{10}O]^+$), 224 ($[M-C_6H_{12}O]^+$), 211 ($[M-C_7H_{13}O]^+$) and 193 ($[M-C_7H_{13}O-H_2O]^+$). The fragmentation pattern also indicates that lachnellulone contains a highly oxygenated molecular nucleus as is the case for lachnelluloic acid.

The high field ^1H NMR spectra of lachnellulone (Fig. 3) demonstrates the presence of two mutually independent spin systems. The first system contains a low field methylene at δ 3.76 (1H, ddd) and δ 3.32 (1H, ddd) (J geminal 20 Hz), which is coupled to another set of methylene protons at δ 2.33 (1H, dddd) and δ 1.87 (2H, dddd) (J geminal 13 Hz). These latter two methylene protons, besides being mutually coupled and coupled to the protons at δ 3.76 and δ 3.32, are coupled to a low-field quintet at δ 5.02 (1H). The low-field proton, which may be geminal to the oxygen of an ester or a lactone, is coupled to four protons: the methylene protons at δ 2.33 and δ 1.87 and δ 1.71. Finally, when the signal at δ 1.87 (2H) is saturated, multiplicity changes take place at δ 5.02, 3.76, 3.32, 2.33, 1.71 and 1.41, thus an aliphatic chain (δ 1.41) is attached to the carbon bearing the two geminal protons at δ 1.87 and δ 1.71. These results suggest the presence of the following partial structure (A').



A'

The second spin system contains a hydrogen bonded hydroxyl proton at δ 3.92 (its chemical shift and coupling pattern are independent of the sample concentration), which appears as a sharp doublet. When this doublet, which is exchangeable with D_2O or CD_3OD, is irradiated a carbinol proton at δ 4.45 (1H, dd) is transformed into a doublet. When the carbinol proton is saturated, another carbinol proton at δ 4.57 (1H, ddd) is affected. Finally, decoupling of the signal at δ 4.57 causes changes in the coupling patterns of the signals at δ 1.71 and δ 1.30, and since the signal at δ 1.30 must arise from a chain of methylenes, it is concluded that the carbon bearing the proton at δ 4.57 is attached to an aliphatic chain. Based on these results the following partial structure (**B'**) is suggested.



B'

The presence of two aliphatic chains in lachnellulone is evident from the signals for two virtually coupled methyl groups at δ 0.96 and δ 0.89, and also from the fact that in the ^1H NMR of lachnellulone there are broad signals at δ 1.42 and δ 1.30, which integrate for 4 and 6 hydrogens, respectively. Such signals are characteristic of saturated chains.

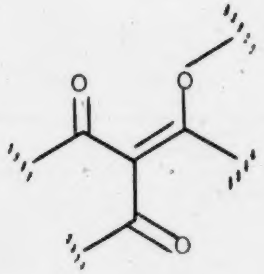
The ^{13}C NMR of lachnellulone shows the presence of 18 carbons in the molecule, four of which are singlet sp^2-hydridized carbons; δ 191 (s), δ 189 (s), δ 165 (s) and δ 100 (s). This supports the hypothesis that the oxygenated portion of lachnellulone is very similar to the one present in lachnelluloic acid, as shown in the following table.

Table VI. ^{13}C NMR chemical shift of sp^2-hydridized carbons in lachnellulone and lachnelluloic acid.

Lachnelluloic Acid δ (ppm)	Lachnellulone δ (ppm)
204.2	191.1
194.4	189.0
163.9	165.3
102.9	100.2

The analysis of the ^{13}C NMR spectra of lachnellulone also indicates the presence of three monooxygenated sp^3 hybridized carbons at δ 91 (d), 77 (d) and 72 (d), as well as nine methylene carbons from δ 36 to δ 23 (all triplets) and two methyl groups at δ 14.1 (q) and δ 14.0 (q).

Consideration of the ^{13}C NMR signal multiplicity indicates the presence of 27 hydrogens directly attached to carbon in lachnellulone. Since this metabolite contains a total of 28 hydrogens (by HREIMS) it is evident that there is only one hydroxyl group in the molecule; that which is the hydroxyl group present in partial structure **B'**. The presence of the two aliphatic chains in lachnellulone is confirmed by the detection of two methyl groups in the ^{13}C NMR spectra. Based on this ^{13}C NMR analysis, plus the other spectroscopic similarities between lachnellulone and lachnelluloic acid, partial structure **C'** is proposed for lachnellulone:

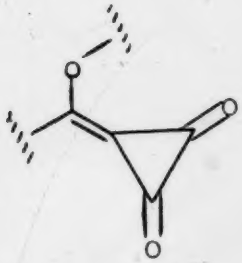


C'

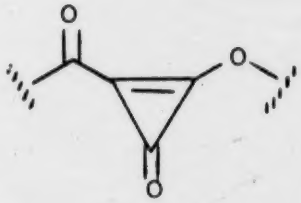
Fragment **C'** in lachnellulone does not bear an enolic proton, as is concluded from the absence of a low field proton (14 to 18 ppm) in the ^{1}H NMR spectra, from the negative result of a ferric chloride test and from the analysis of the ^{13}C NMR signal multiplicity, which shows the presence of only one alcoholic hydroxyl group in the molecule. Thus, fragment **C'** must contain an enol ether as one of the four points of substitution.

The molecular formula assigned for lachnellulone indicates that this natural product contains five unsaturations or unsaturation equivalents. Fragment **C'** accounts for three of those unsaturations, and since the ^{13}C NMR spectra of lachnellulone does not show signals for more sp^2 hybridized carbons than the ones present in fragment **C'**, it is concluded that the last two unsaturations correspond to two rings. Thus, lachnellulone is a bicyclic compound.

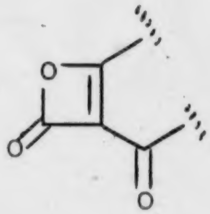
The four free attachment points of fragment **C'** cannot be joined with one another, because in such a case either a three-membered ring α-diketone (a), a cyclopropenone (b) or an unsaturated β-lactone (c) would be produced. These highly strained functionalities are clearly not present in lachnellulone.



a



b

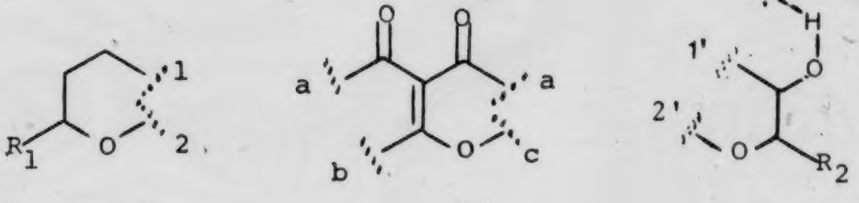


c

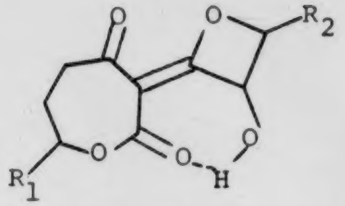
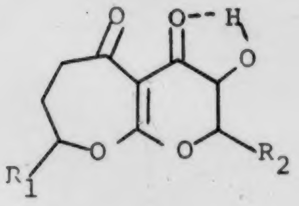
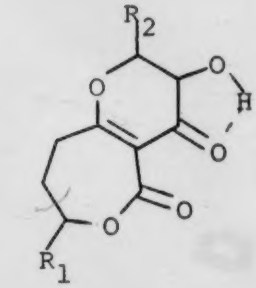
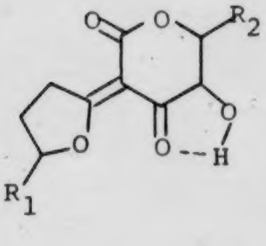
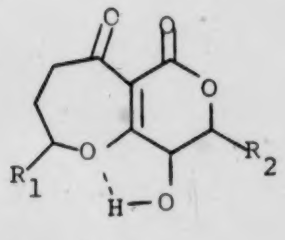
Two of the free attachment points of fragment **C'** must be attached to the two free attachment points of fragment **A'**, forming one of the rings present in lachnellulone. The other two ends of fragment **C'** must be linked to the two attachment points of fragment **B'**, thus forming the second ring present in lachnellulone.

From HREIMS it is known that lachnellulone contains only five oxygen atoms, but by adding the number of oxygen atoms of fragments **A'**, **B'** and **C'**, the total is six oxygen atoms. This implies that either the ethereal oxygen atom on fragment **A'** or the one on fragment **B'** is the same as the enol ether oxygen in fragment **C'**. With these limiting conditions there are only five different ways of assembling the three fragments of lachnellulone. These are shown in the following scheme.

SCHEME 6:


1
2
R1
O
a
a
b
c
1'
2'
H
O
R2

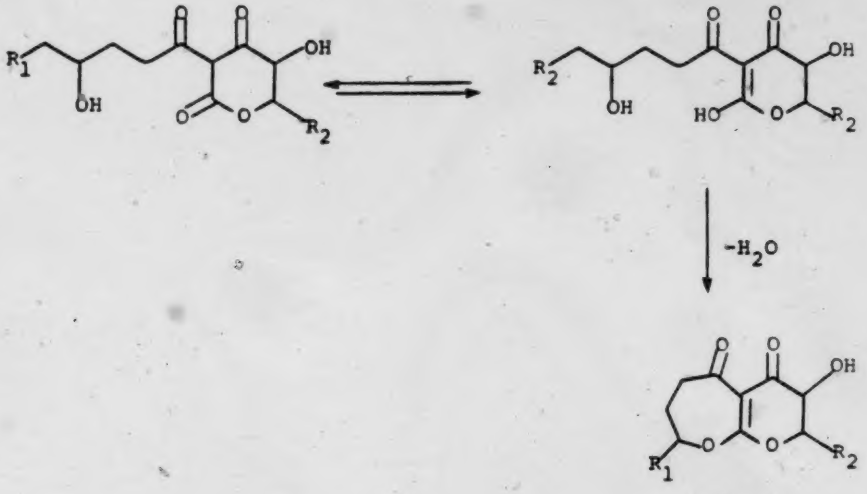
A' **B'** **C'**

		JOINING	
I		1-a 2-a	1'-b 2'-c
II		1-a 2-c	1'-a 2'-b
III		1-b 2-a	1'-a 2'-c
IV		1-b 2-c	1'-a 2'-a
V		1-a 2-c	1'-b 2'-a

Structures I and IV were considered to be unlikely candidates for the structure of lachnellulone. These two structures do not explain the spectroscopic characteristics of lachnellulone.

Structure II was rejected as the structure of lachnellulone for three main reasons.

1. The formation of structure II suggests that enolization of a lactone carbonyl takes precedence over enolization of a ketone in the possible intermediates, or that the least electrophilic lactone carbonyl undergoes preferential nucleophilic cyclization.

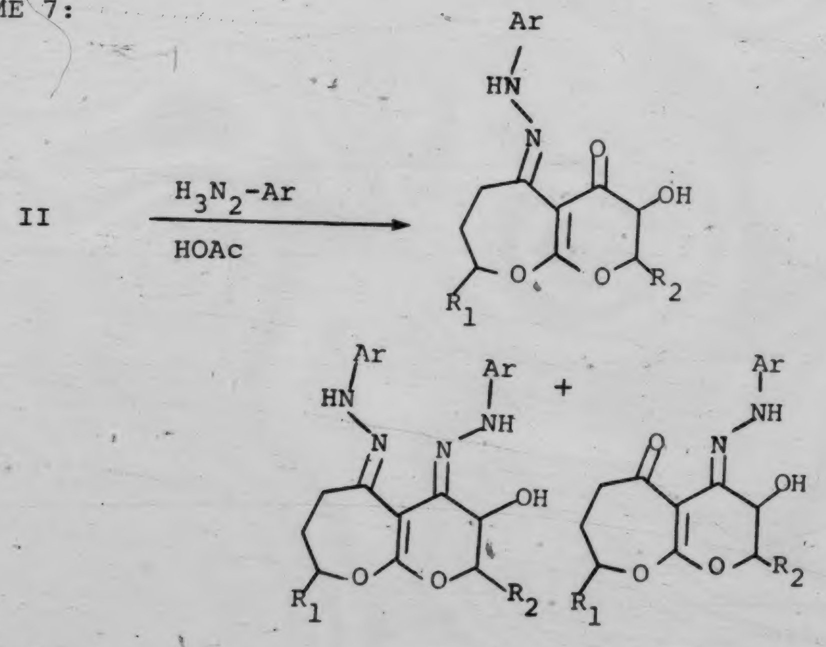


The enolic form in structure II does not correspond to the one present in lachnelluloic acid, as should be the case since the spectral details (UV, IR, ^{13}C NMR) of both compounds are quite similar.

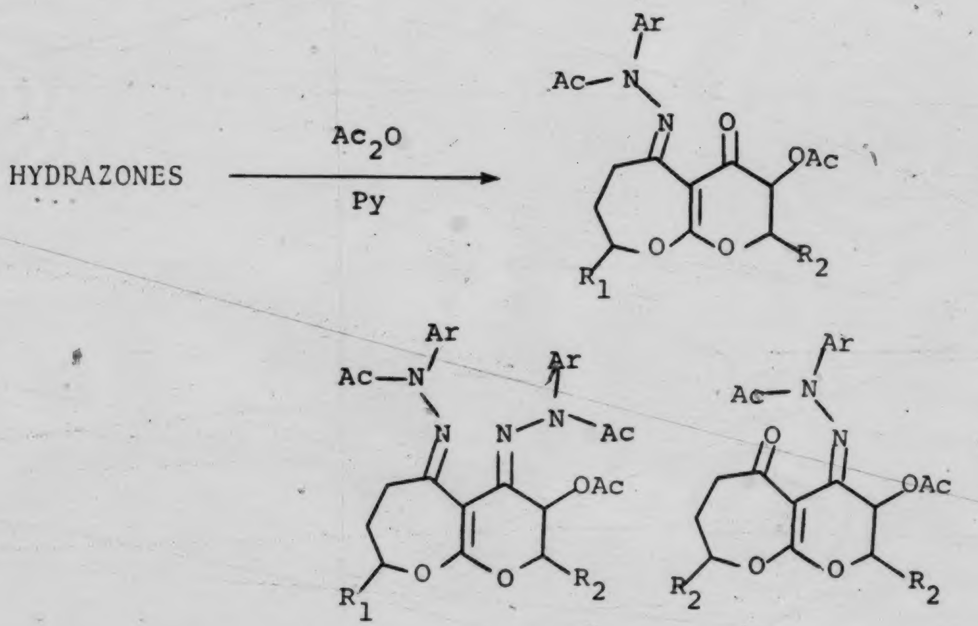
2. When lachnellulone is treated with p-nitrophenylhydrazine a monohydrazone is formed (^{1}H NMR Fig. 5).[24] This result was confirmed by acetylation of the hydrazone of lachnellulone to give a diacetyl derivative (^{1}H NMR Fig. 6). A diacetate is formed because both the hydroxyl group present in lachnellulone and the hydrogen bearing nitrogen of the hydrazone are acetylated.

Structure II would be expected to form either two monohydrazones or, a mixture of monohydrazones and a dihydrazone, as shown in the following scheme.

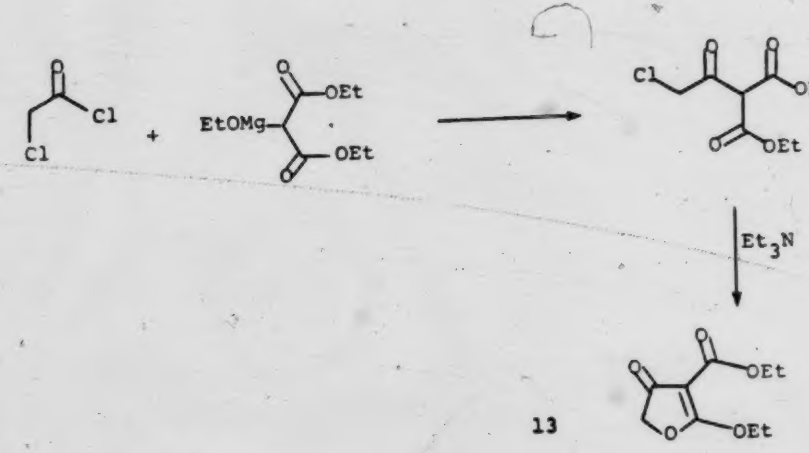
SCHEME 7:



SCHEME 7 (cont.)

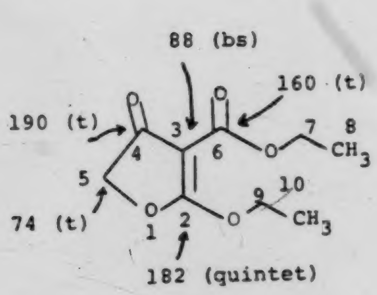

HYDRAZONES
Ac2O
Py
Ar
Ac
N
O
OAc
R1
R2

3. The ^{13}C NMR shifts of model compound **13**, prepared using reported methodology,[25] do not correspond to the chemical shifts in the ^{13}C NMR spectrum of lachnellulone.


Cl
Cl
+
EtOMg
OEt
OEt
Cl
OEt
OEt
Et$_3$N
OEt
OEt

13

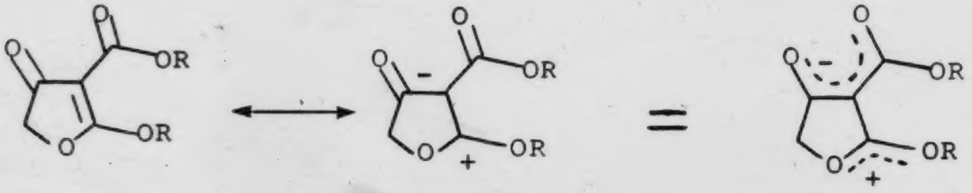
The gated decoupled ^{13}C NMR spectra for this model compound clearly indicates the following ^{13}C NMR assignment (see Experimental section).


88 (bs)
160 (t)
190 (t)
3
7
8
CH$_3$
4
6
5
9
10
74 (t)
O
1
2
O
CH$_3$
182 (quintet)

13

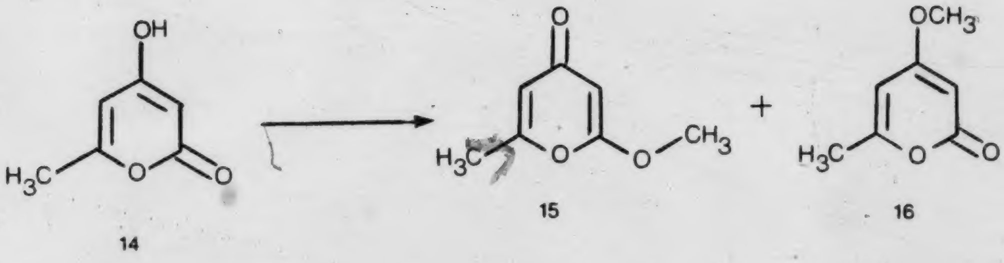
The ^{13}C NMR assignment of C-2 and C-3 in the model compound (δ 182 and δ 88, respectively) does not correspond to the assignment of the respective carbons in structure II (δ 165 and δ 100). Since lachnellulone does not exhibit ^{13}C NMR absorptions that correspond to the values observed in the model compound, structure II is not lachnellulone.

The somewhat anomalous chemical shifts for C-2 and C-3 in the model compound can be explained on the basis of the high polarization of the carbon-carbon double bond, which leaves a high electronic density at C-3 and a low electronic density at C-2.



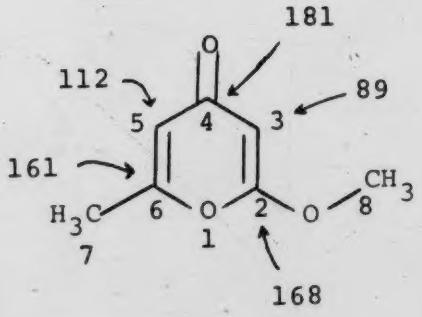
The UV spectra of the furan derivative (**13**) (λ_{max} 222, 248 nm) is significantly different from that of lachnellulone (λ_{max} 218, 275 nm).

Another model compound (**15**) was prepared by treatment of triacetic acid lactone (**14**) with diazomethane (see part II, page 136).[26]


OH
H_3C
O
O
+
O
H_3C
O
O
CH_3
OCH_3
H_3C
O
O

14 15 16

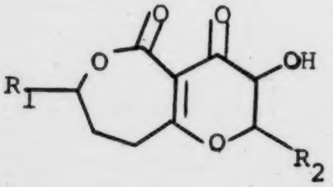
The ^{13}C NMR assignment of compound **15** was obtained by gated decoupling techniques and is shown below.


181
112
89
O
5
4
3
161
CH_3
H_3C
6
O
2
O
8
7
1
168

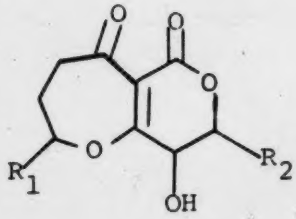
15

These results also indicate that this kind of moiety (an sp^2 carbon bearing two oxygens) is not present in lachnellulone. The most indicative result is the observed chemical shift of the electron rich C-3, which is absent in lachnellulone.

Having eliminated three of the possible structures for lachnellulone, only two possibilities remain, structure III and structure V.



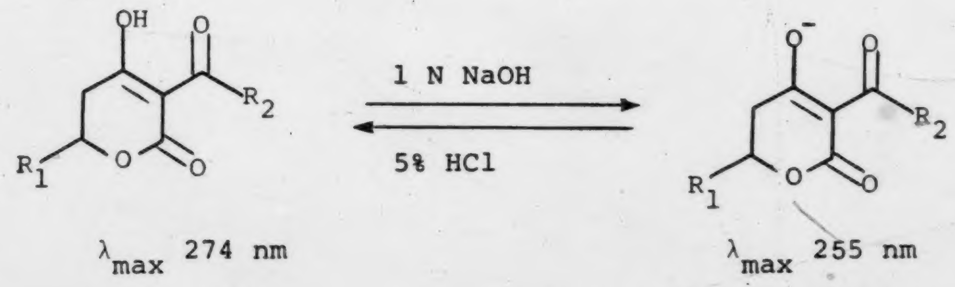
III



V

Both structures can explain the formation of a monohydrazone from lachnellulone, as well as the observed ^{13}C NMR and UV behaviour. However, there is a detail in the ^{1}H NMR spectra of lachnellulone that may not be explained by structure V: an alcoholic hydroxyl group which is strongly hydrogen bonded. The chemical shift for this hydroxyl proton appears at δ 3.92. It is independent of the concentration of the sample and to a certain extent on the solvent used. This observation can only be explained by the presence of an intramolecular hydrogen bond between the hydroxyl group and one of the carbonyls. The possibility of having this type of intramolecular hydrogen bond in structure V is very remote, but structure III easily accommodates such a demand (see page 62).

When lachnellulone is treated with dilute sodium hydroxide in methanol, its UV spectra undergoes a hypsochromic shift (274 nm to 255 nm). This same phenomenon occurs with lachnelluloic acid as was mentioned before (see table III. Since in both cases the effect of the base is reversed by addition of 5% HCl it was concluded that the change occurring in the chromophore is reversible. For lachnelluloic acid this hypsochromic shift is rationalized as follows.

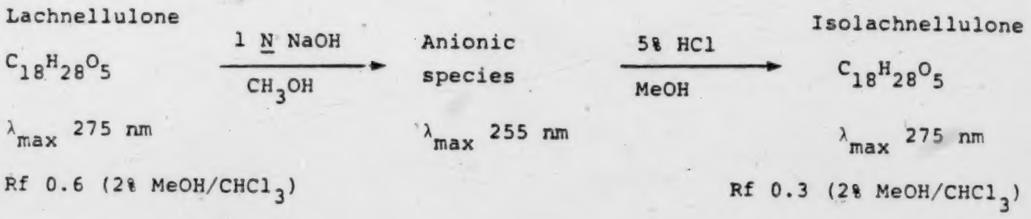


Thus, lachnellulone must have a structure which allows the formation of an anionic species similar to that of lachnelluloic acid (**6**) when it is treated with sodium hydroxide.

After treatment of lachnellulone (10 mg) with 1 M NaOH followed by acidification, a new compound, different from lachnellulone, was isolated. This compound has an Rf of 0.3 in 2% MeOH in $CHCl_3$ (the Rf of lachnellulone in this solvent is 0.6). The compound was easily purified by flash chromatography to afford 4.8 mg of a product which we call isolachnellulone.

It seems clear that the anionic species formed when lachnellulone is treated with aqueous base is converted to isolachnellulone by treatment with acid, and that isolachnellulone exhibits the same UV spectra as lachnellulone. This is summarized in the following scheme.

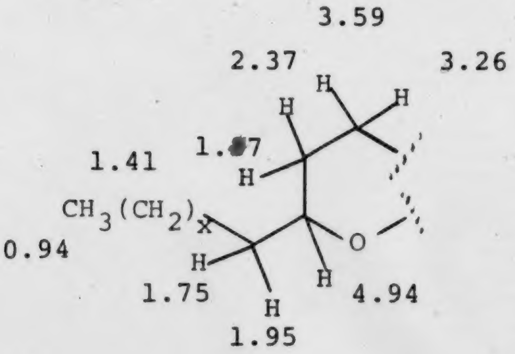
SCHEME 8:


Lachnellulone
$C_{18}H_{28}O_5$
λ_{max} 275 nm
Rf 0.6 (2% MeOH/$CHCl_3$)
1 N NaOH
CH_3OH
Anionic species
λ_{max} 255 nm
5% HCl
MeOH
Isolachnellulone
$C_{18}H_{28}O_5$
λ_{max} 275 nm
Rf 0.3 (2% MeOH/$CHCl_3$)

The HREIMS of isolachnellulone shows that it is isomeric with lachnellulone because it has the same molecular weight and the same molecular formula (MW 324 ($C_{18}H_{28}O_5$)). However, the fragmentation pattern of isolachnellulone is different.

The high resolution ^1H NMR spectrum (Fig. 4) indicates that isolachnellulone is in fact an equimolecular mixture of four very closely related compounds which are inseparable by chromatography. Since most of the ^{1}H NMR signals of these four compounds overlap exactly, with the exception of the two signals used to calculate the relative ratios, these four compounds must be diastereoisomers.

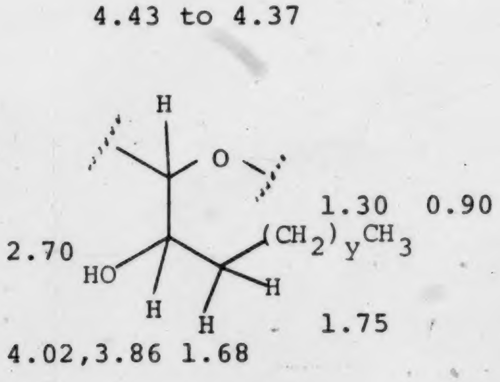
Two isolated spin systems are observed in the ^{1}H NMR spectra of isolachnellulone. One of those spin systems is also present in lachnellulone and it corresponds to the partial structure **A'** in the natural product.



A'

Fragment **A'** contains a geminal pair of protons (δ 3.59 (1H, m) and δ 3.26 (1H, m)) which are coupled to a second geminal pair (δ 2.37 (1H, m) and δ 1.87 (1H, m)). This last geminal pair is coupled to a low field multiplet (δ 4.94 (1H, m)) which in turn is coupled to two methylene protons (δ 1.95 and δ 1.75). Saturation of these two methylene protons shows that they are part of an aliphatic chain.

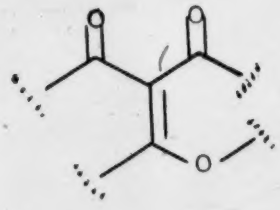
The second spin system contains a one proton multiplet which was shown to correspond to four doublets by multiple irradiation experiments. This indicates the presence of four compounds in the sample. The four doublets (δ 4.43, 4.41, 4.39 and 4.37) are coupled to two multiplets at δ 4.02 and δ 3.86 the intensities of which correspond to one proton. These two multiplets, which are simplified by D_2O or CD_3OD exchange, are coupled to a broad doublet (δ 2.70, -OH, exchanges with CD_3OD) and a methylene pair (δ 1.75 and δ 1.68) which forms part of an aliphatic chain. Based upon these results, partial structure B" is suggested.



B"

The epimerization of the two asymmetric centers in this fragment explains the presence of four diastereoisomers of isolachnellulone.

Since the UV spectrum of isolachnellulone is the same as the UV spectrum of lachnellulone it is logical to assume that the same chromophore is present in both substances, that is, both compounds contain the same highly oxygenated molecular nucleus **C'**.

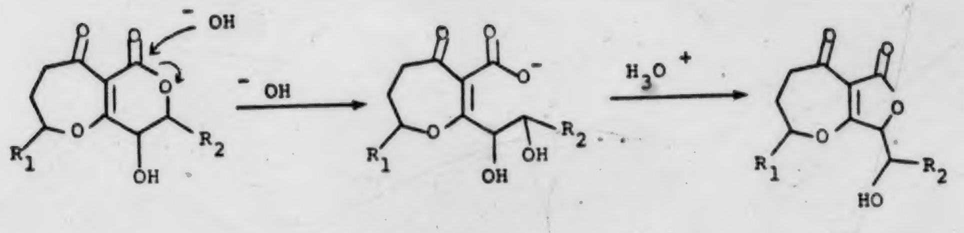


C'

From these results it is clear that only partial structure **B'** of lachnellulone, i.e., that containing the hydroxyl group, was modified by the treatment with base to afford isolachnellulone.

This isomerization can be explained using either structure V or structure III. If lachnellulone has structure V, a lactone hydrolysis would be necessary to explain the transformation. If structure III depicts lachnellulone, a 1,4- addition would be necessary to explain the isomerization. This is summarized in the following Scheme.

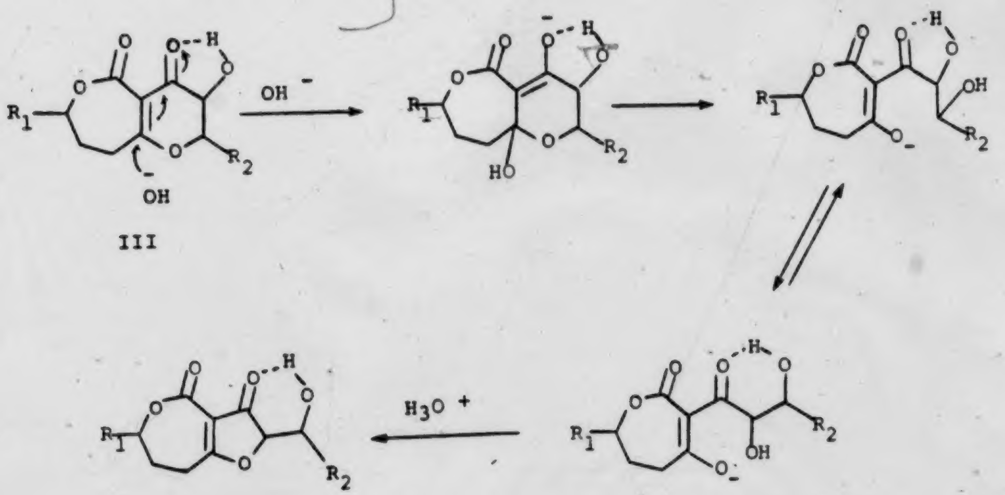
SCHEME 9 :



- OH
- OH
H_3O^+
R1
R2
OH

V

VI



III

VII

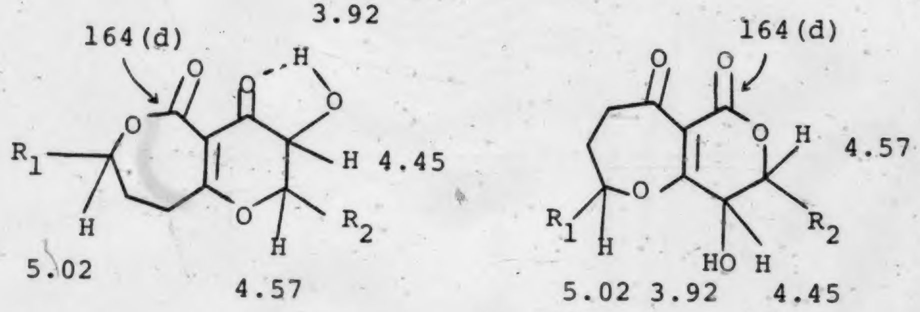
In order to differentiate between structures III and V the gated decoupled spectrum of lachnellulone was recorded, and the multiplicity of the sp^2-hybridized carbons analyzed. The results are shown in Table VII.

Table VII. Long range coupling of the sp^2-hybridized carbons in lachnellulone

δ (ppm)	multiplicity	J (Hz)
191	dt	10.0,2.5
188	m	4.0
164	d	8.0
100	s	-

The signal at δ 164 (d) corresponds to the lactone carbonyl carbon in either III or V. Both structures, III and V, can explain the multiplicity of this carbon (doublet). However, the proton responsible for the multiplicity of the lactone carbonyl carbon would differ in structures III or V. If lachnellulone possesses structure III the proton coupled to the carbon at δ 164 would be the one that resonantes at δ 5.02 in the ^1H NMR spectra of the natural product. On the other hand, if structure V corresponds to lachnellulone, the proton coupled to the carbon at δ 164 would be the one that resonates at δ 4.57 in the ^1H NMR spectrum of lachnellulone. This is illustrated in Scheme 10.

SCHEME 10:



III   V

It is possible to differentiate between structures III and V using selective carbon-hydrogen decoupling experiments. If irradiation at δ 5.02 in the ^1H NMR spectrum of lachnellulone converts the signal at δ 164 into a singlet, lachnellulone would have structure III. However, if saturation at δ 4.57 transforms the signal at δ 164 in the gated ^{13}C NMR spectrum of lachnellulone into a singlet, the structure of lachnellulone is V. The results of several selective decoupling experiments are shown in Table VIII.

Table VIII. Selective decoupling results for lachnellulone.

Carbon δ (ppm)	Multiplicity (J in Hz)	Decoupled at δ5.02*	Decoupled at δ4.57*	Decoupled at δ3.32*
191	dt(10,2.5)	bt	s	dt
188	m(4)	m(4)	m(4)	bt
164	d(8.0)	d(4)	s	d(8)
100	s	-	-	-

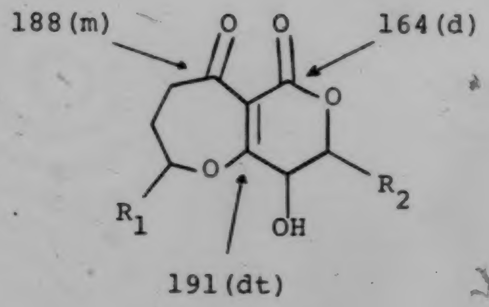
*Clean decoupling experiments are not possible, since protons within a ± 0.5 ppm range are also affected.

When the gated decoupled spectrum of lachnellulone is run in $CDCl_3$ with CD_3OD, the multiplicity of the signal at δ 191 changes from a doublet of triplets to a doublet of doublets.

It seems clear from Table VIII that the structure of lachnellulone is structure V, since the doublet at δ 164 in the gated ^{13}C NMR spectrum of the natural product collapses when the signal at δ 4.57 (and δ 4.45) is saturated. Furthermore, saturation at δ 5.02 only induces a decrease of the coupling constant of the signal at δ 164.

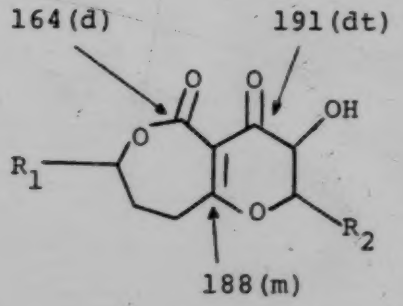
Assuming that lachnellulone possesses structure V, the enol ether carbon would resonate at δ 191 since it is the closest sp^2 carbon to the alcoholic OH, and it is the signal at δ 191 which changes multiplicity when the hydroxyl proton is exchanged for deuterium. In addition, the signal at δ 191 is the only signal whose multiplicity is affected when either the proton at δ 5.02 or the one at δ 4.57 (and δ 4.45) are decoupled. The irradiation of the methylene proton at δ 3.32 induces a multiplicity change in the carbon that resonates at δ 188, therefore, this carbon corresponds to the ketone carbonyl carbon. This ^{13}C NMR assignment is shown in Scheme 11 for structure V.

SCHEME ~~11:~~



On the other hand, if we assume that lachnellulone possesses structure III, it is difficult to explain why the signal at δ 164 collapses to a singlet when the proton at δ 4.57 (and δ 4.45) in the ^1H NMR spectrum of lachnellulone is irradiated. In addition, if an explanation for the behavior of the lactone carbonyl carbon is found, it still would be necessary to explain why the multiplicity of the signal at δ 188 changes when the proton at δ 3.32, and not the proton at δ 4.57, is saturated. Based on the results on Table VII the ^{13}C NMR assignment for structure III would be as shown in Scheme 12.

SCHEME 12:

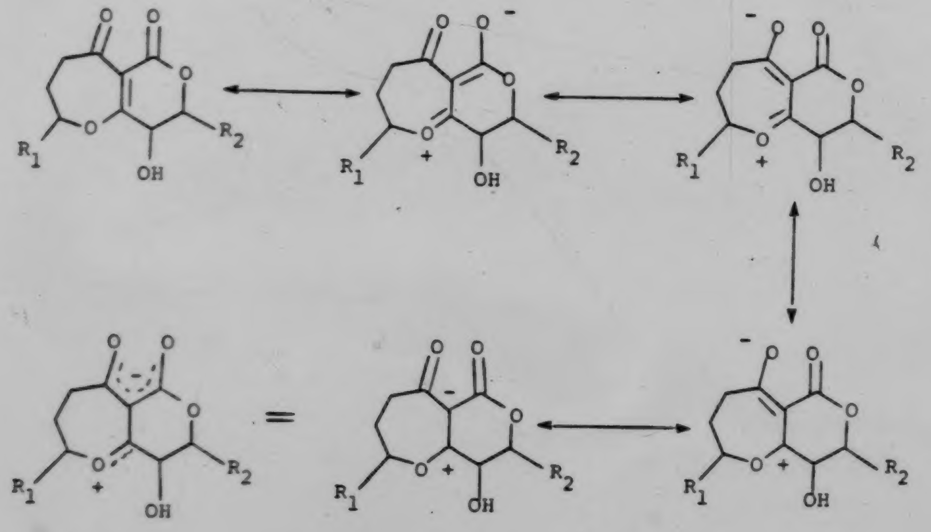


Thus, the gated decoupled and selective decoupled spectra of lachnellulone strongly suggest that the structure of the natural product is structure V rather than structure III.

Lachnellulone displays a strongly hydrogen bonded hydroxyl absorption at δ 3.92 (d). Since the chemical shift of this proton does not vary with the sample concentration, and to a certain degree with the solvent used, it is an intramolecular hydrogen bond.

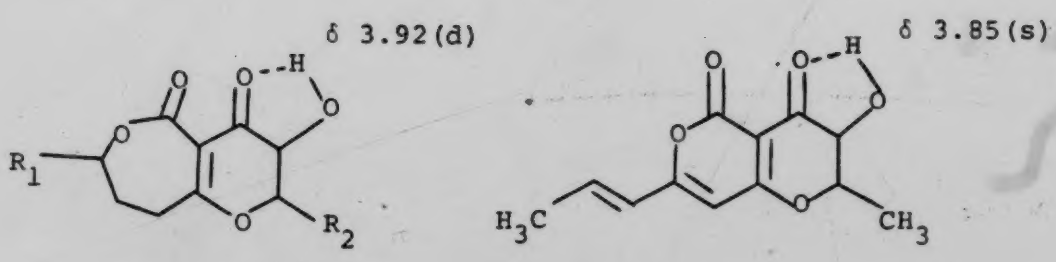
As mentioned before, structure V for lachnellulone cannot explain such an intramolecular hydrogen bond, since the only oxygen atom that is close enough to the alcoholic hydroxyl, is probably an electropositive oxygen. This ether oxygen is in conjugation with two carbonyls, therefore its electronic density must be lower than that of a normal ether oxygen. In addition, it is the presence of this relatively electropositive oxygen that explains the unusual chemical shift of the carbons at δ 91 (d) and δ 191 (dt) in the ^{13}C NMR spectra of lachnellulone. This is shown in Scheme 13.

SCHEME 13:



On the other hand, structure III explains easily the hydrogen bonded hydroxyl group of lachnellulone, as shown in Scheme 14.

SCHEME 14:



III

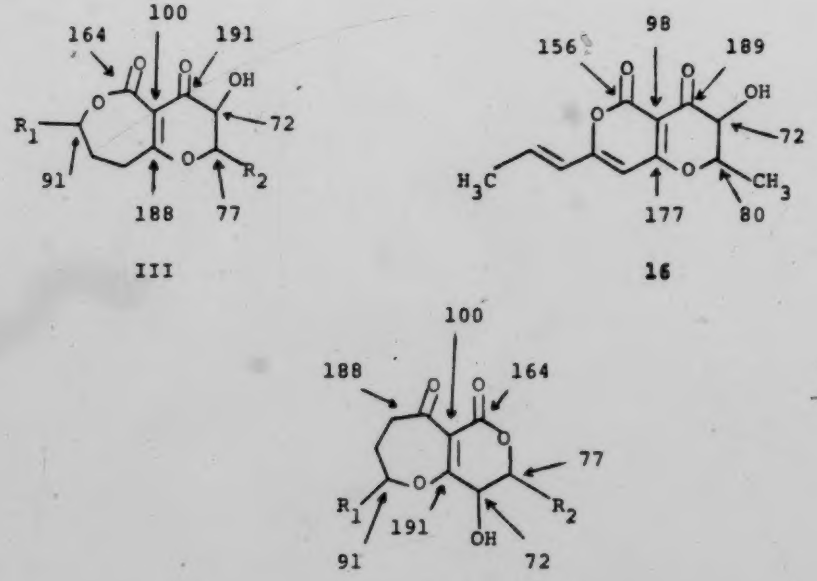
16

And as can be seen in Scheme 14 the antibiotic radicinin (**16**),[27] the structure of which resembles structure III, possesses a similarly located hydroxyl group, with relatively the same chemical shift as the hydroxyl proton in lachnellulone.

The presence of this intramolecularly hydrogen bonded hydroxyl group in lachnellulone clearly favors structure III over structure V as the structure of lachnellulone.

The resemblance between radicinin (**16**) and lachnellulone involves more than just the hydrogen bonded hydroxyl. The ^{13}C NMR spectrum of radicinin is similar to the ^{13}C NMR spectrum of lachnellulone, and although this similarity can be explained using either structure III or V, structure III explains it better, as shown in Scheme 15.

SCHEME 15:


100
164
191
OH
72
R1
91
188
77
R2
98
156
189
OH
72
H3C
177
80
CH3
100
188
164
77
R1
191
R2
OH
91
72

III

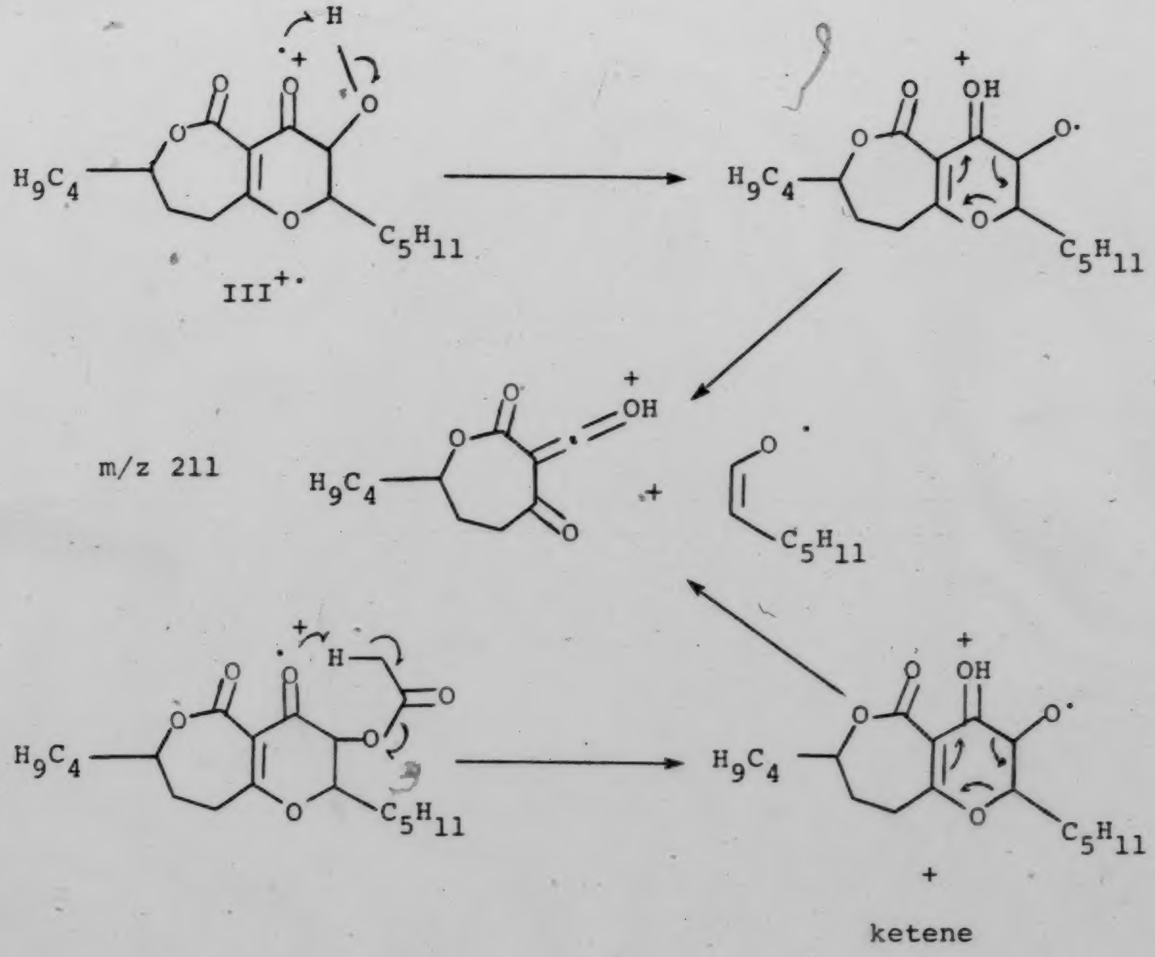
16

V

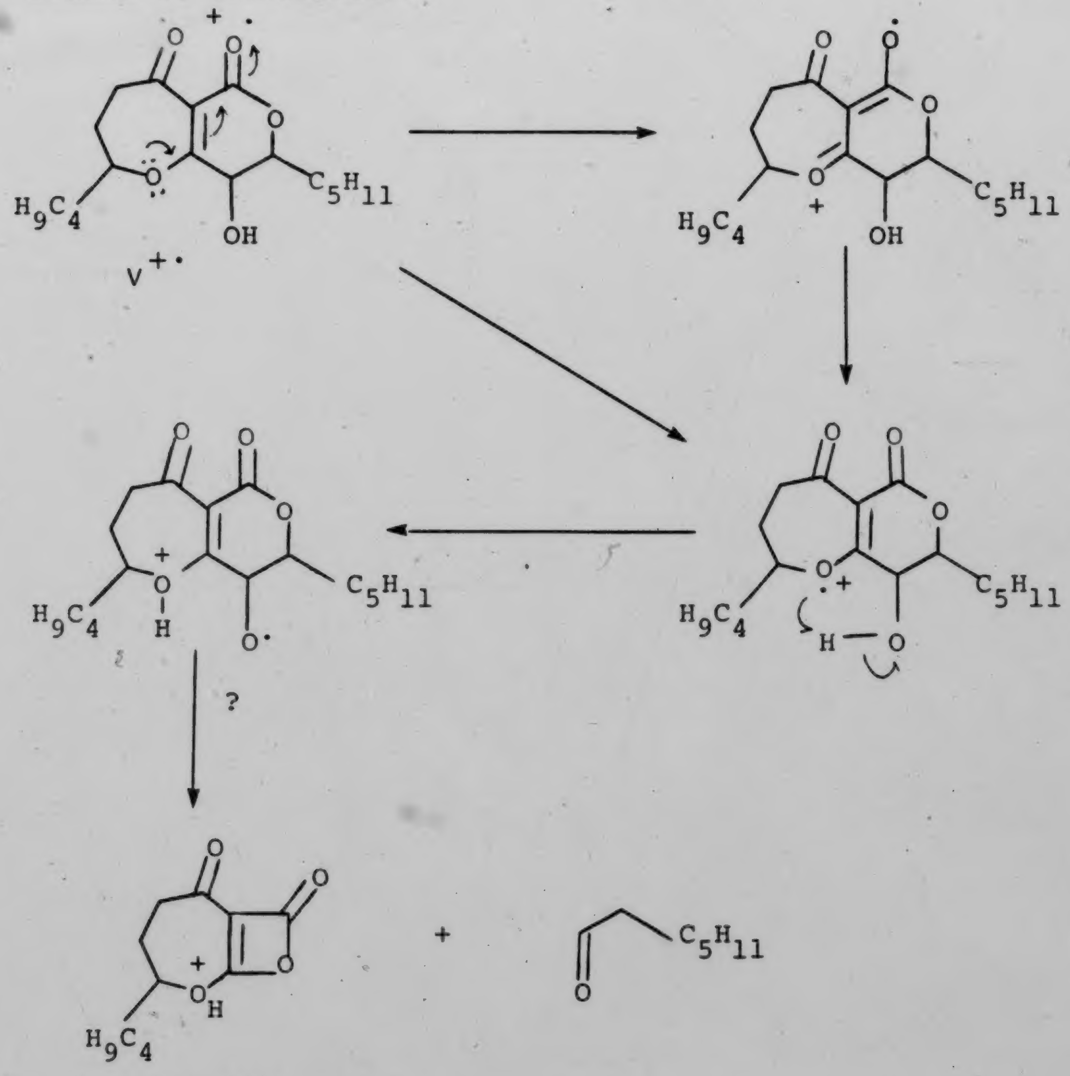
The HREIMS of lachnellulone shows as the most intense fragment m/z 211 ($C_{11}H_{15}O_4$) which is formed by loss of $C_7H_{13}O$ from the molecular ion. The oxygen atom lost in this fragmentation was shown to be the hydroxyl oxygen, since lachnellulone monoacetate gives the same intense fragment m/z 211 ($C_{11}H_{15}O_4$).

This fragmentation can be explained assigning as R_1 - C_4H_9 and as R_2 - C_5H_{11} for either structure III or V, as shown in Scheme 16.

SCHEME 16:


H
O
O
O
O
H_9C_4
C_5H_{11}
$III^{+\cdot}$
+
OH
O
O·
H_9C_4
C_5H_{11}
m/z 211
+
OH
O
O
O
H_9C_4
+
O
C_5H_{11}
H
O
O
O
O
O
H_9C_4
C_5H_{11}
+
OH
O
O·
H_9C_4
C_5H_{11}
+
ketene

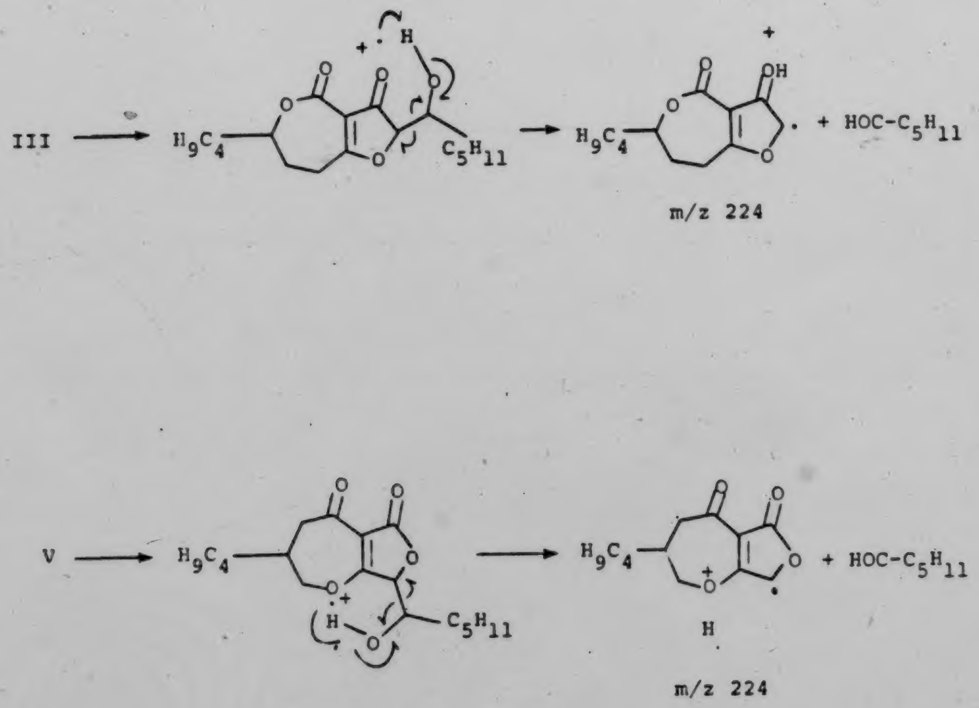
SCHEME 16 (continued):



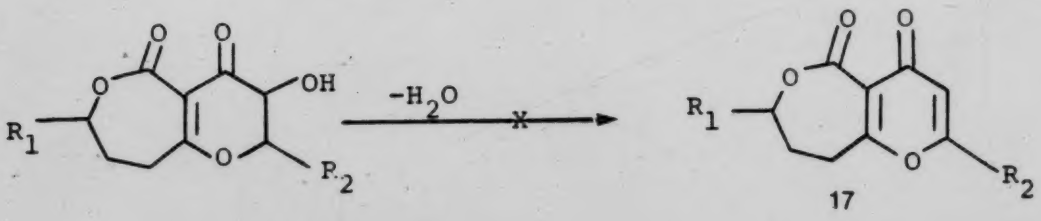
However we feel that this fragmentation is explained in a more satisfactory way using structure III.

In a similar way, the most abundant fragment for isolachnellulone can be explained more convincingly using structure III, as shown in Scheme 17.

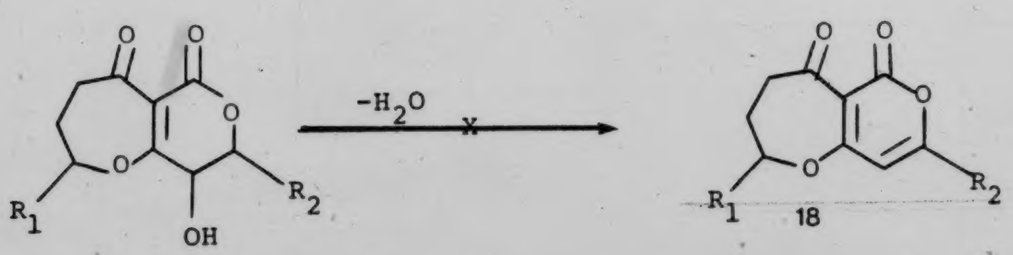
SCHEME 17:


III
H9C4
C5H11
m/z 224
+ HOC-C5H11
V
H9C4
C5H11
m/z 224
+ HOC-C5H11

A possible way to assign either structure III or structure II to lachnellulone is to dehydrate the natural product. If lachnellulone possesses structure III dehydration would afford the 4-pyrone **17**, but if structure V is lachnellulone the dehydration reaction would give the 2-pyrone **18.** Compounds **17** and **18** could be differentiated by their UV spectra or their coupled ^{13}C NMR. Attempts to dehydrate lachnellulone failed using either $SOCl_2$ or $POCl_3$ in pyridine at room temperature and at 0°C.



17

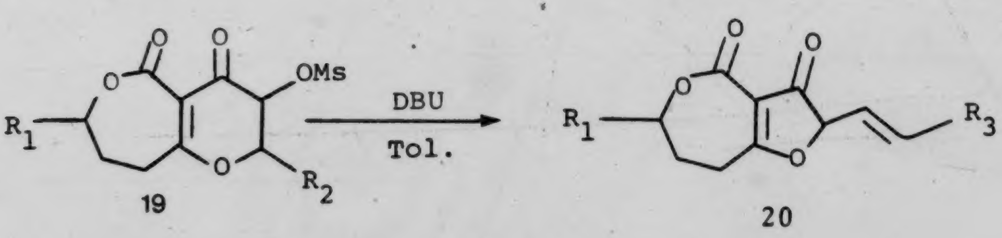


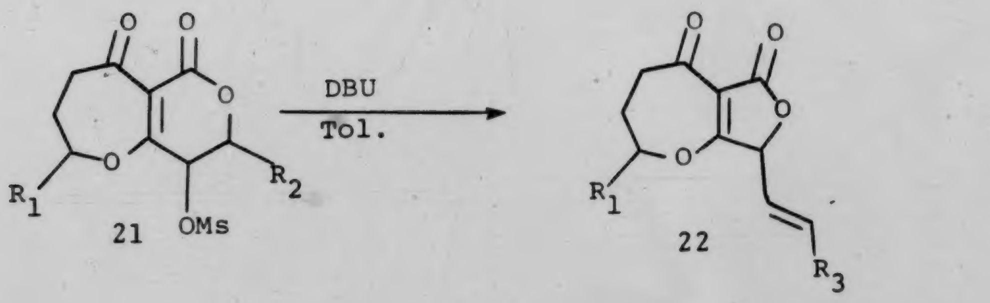
18

Instead, very complex reaction mixtures were obtained.

Lachnellulone was converted to its mesyl ester (**19** or **21**) by treatment with MsCl and pyridine in CH_2Cl_2 at room temperature. The mesylate was obtained in quantitative yield (MW 402 ($C_{19}H_{30}SO_7$), ^1H NMR δ 5.12 (1H, d), 4.96 (1H, m), 4.48 (1H, m).

Elimination of the mesyloxy group on treatment with DBU in toluene at room temperature did not give **17** or **18**, but afforded a product containing a disubstituted carbon-carbon double bond; ^1H NMR δ 5.92 (1H, dt), 5.52 (1H, dd), 4.48 (1H, bd), which we believe possesses structure **20** or **22**. Further experimentation is necessary to confirm this assumption.



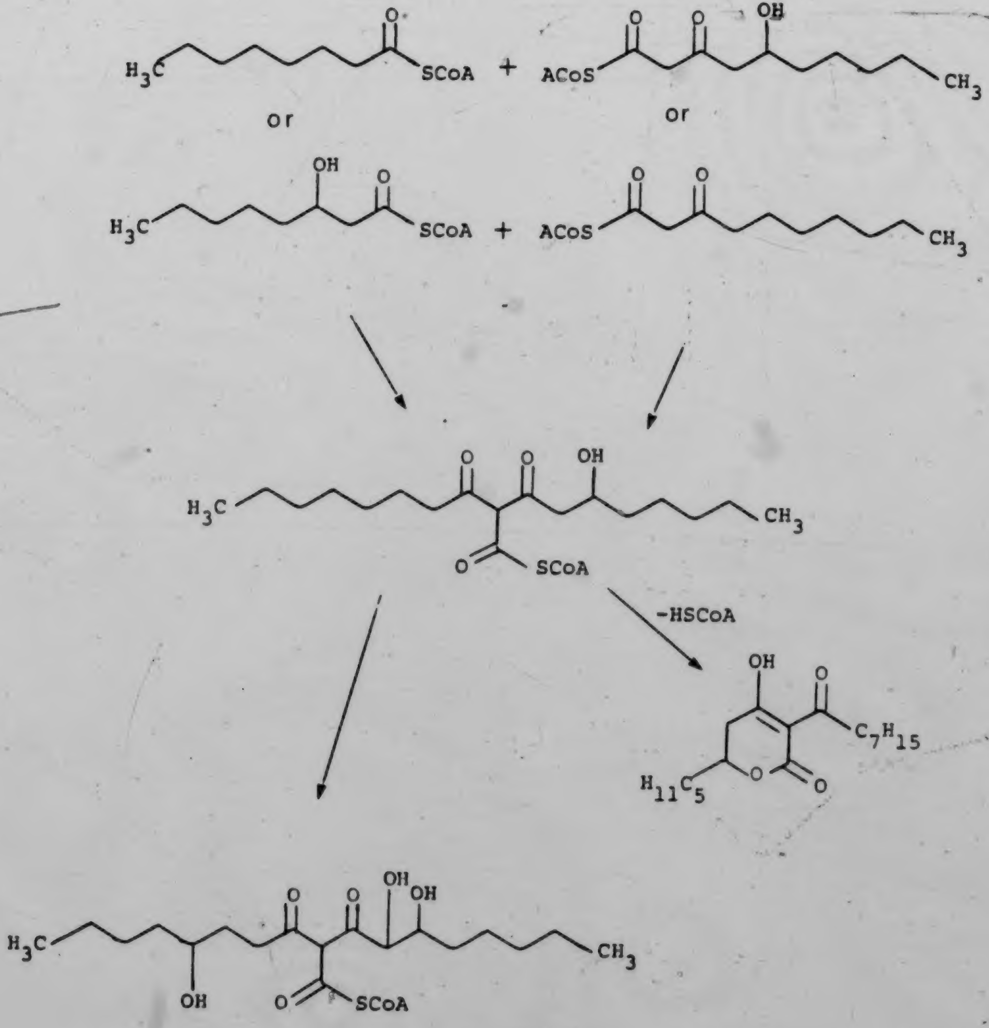


Lachnellulone was also treated with Jones reagent at room temperature, but a complex mixture of products was obtained.

Further experimentation is necessary to assign the final structure for lachnellulone. In the near future an X-ray diffraction experiment will be used to make the final decision. We feel that at the present time it is not possible to distinguish between the two possible structures for lachnellulone.

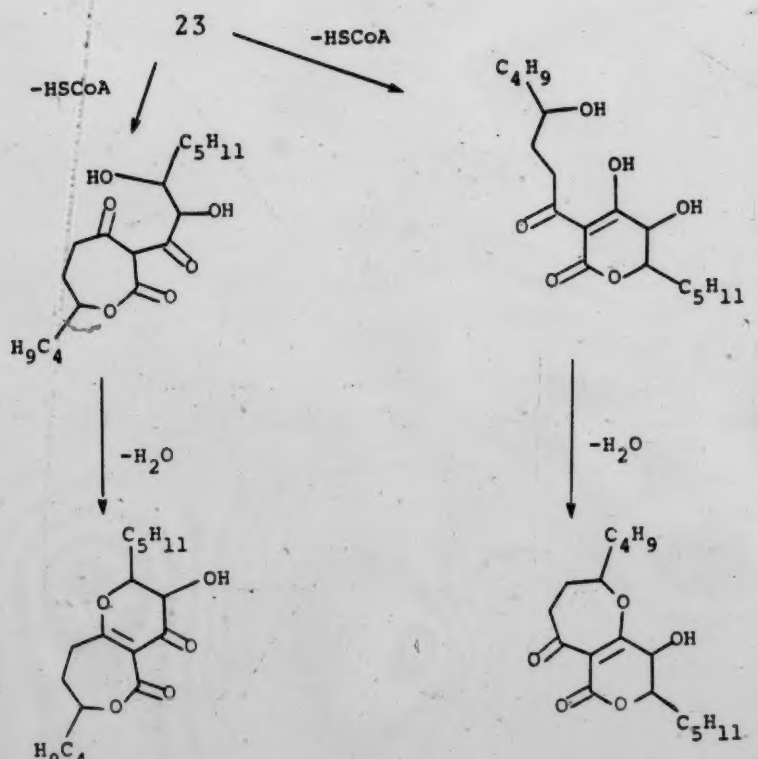
Biogenetically both structures III and V could be related to lachnelluloic acid. We believe that they may derive from the condensation of two different polyketide chains, as shown in the following scheme.

SCHEME 18:


H3C
SCoA
+
ACoS
OH
CH3
or
or
H3C
OH
SCoA
+
ACoS
CH3
H3C
OH
CH3
SCoA
-HSCoA
OH
C7H15
H11C5
O
H3C
OH OH
CH3
OH
SCoA

23

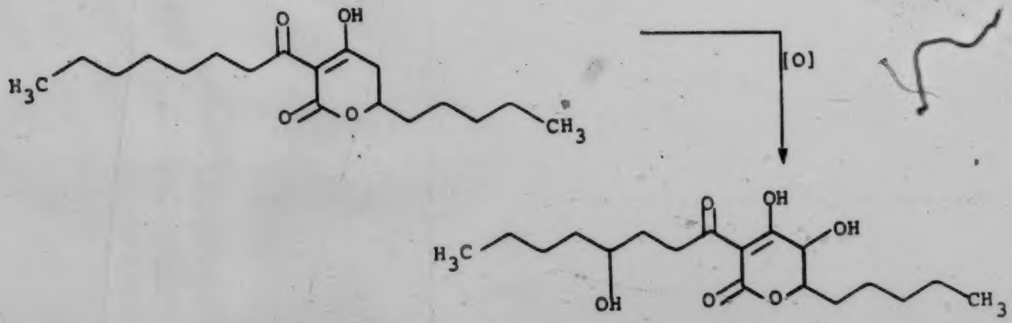
SCHEME 18 (cont.)


23
-HSCoA
-HSCoA
-H$_2$O
-H$_2$O

III

V

The monocyclic intermediate to structure V may also be derived from lachnelluloic acid by oxidation.



The nitrogen containing metabolite

From the fractions obtained by eluting the flash column of the crude mycellium extract with 10% MeOH in $CHCl_3$, a white solid was obtained by trituration with dichloromethane. This compound was recrystallized from hot methanol to afford white crystals (mp 140-142°C) which were insoluble in chloroform or ethyl acetate.

The FTIR of this solid showed sharp absorptions at 3340 and 3200 cm^{-1} (-N-H), 1740 cm^{-1} (-COOR), 1620 cm^{-1} (C=C) and 720 cm^{-1} ($-CH_2-$ rocking).

The HREIMS shows as the heaviest fragment m/z 683 ($C_{42}H_{85}NO_5$, one unsaturation), and fragmentations for the loss of one and two molecules of water.

The ^1H NMR results (Fig. 7) indicate the presence of two aliphatic chains since there are two virtually coupled methyl groups (δ 0.90 (6H, vt)). This molecule contains six hydrogens geminal to oxygen or nitrogen at δ 5.14, 4.65, 4.55, 4.45, 4.39 and 4.31; and three D_2O exchangeable protons at δ 6.72 (2H) and 6.23 (1H).

The structure of this compound has not yet been elucidated. It is present in small amounts (5 mg or less from 10 ℓ of ferment) or not at all in the crude extract. This compound is pharmacologially inactive.

The lachnelluloic acid analogue T-1.

Fractions obtained by eluting the flash column of the crude mycelium extract of L. fuscosanguinea with 2% MeOH in $CHCl_3$ contain a white solid insoluble in most organic solvents. The purification of this compound was accomplished by washing the dust-like crystals several times with ethyl ether. These white crystals have a high melting point (239-240°C) and are soluble in pyridine.

The FTIR of the white compound which is called T-1 shows the presence of a hydroxyl group (3420 cm^{-1}), an α,β unsaturated carbonyl (1660 cm^{-1}, C=O, 1610 cm^{-1}, C=C) and a chelated carbonyl (1590 cm^{-1}).

The UV spectra in methanol of this compound show absorption maxima at λ 241 nm and 273 nm. The first absorption maximum (241 nm) shifts to 250 nm in the presence of sodium hydroxide and to 219 nm in the presence of acid.

Surprisingly, the ^1H NMR spectra of compound T-1 in either DMSO-d_6 or pyridine-d_5 (Fig. 8) is almost identical with that of lachnelluloic acid; δ 4.10 (1H, m), 3.39 (m, H_2O), 2.71 (1H, m), 2.31 (1H, m), 1.58 (1H, m), 1.45 (1H, m), 1.3-1.2 (16H, m) and 0.90 (6H, vt).

The HREIMS of compound T-1 shows the peaks obtained for lachnelluloic acid, while a fast atom bombardment (FAB) experiment shows that the actual molecular weight of this molecule is greater than 310 (lachnelluloic acid). Information regarding its molecular weight were inconclusive.

Due to the very small amounts of compound T-1 isolated, neither its structure nor its biological activity has been determined.

Fermentor extract.

TLC studies of the crude mycelium extract from the fermentor show the presence of four major compounds. Flash chromatography of this extract affords a mixture of triglycerides (least polar fractions) and a mixture of three other major compounds.

Gel permeation chromatography of the medium polarity fractions with Sephadex LH-20 afforded a yellow compound, which was recrystallized from chloroform. The yellow crystalline dye was found in very small quantities and its structure has not been elucidated (^{1}H NMR Fig. 9). Other fractions from the Sephadex LH-20 column contain the remaining two major components of the mycelium extract. The TLC analysis, which shows two overlapping compounds, indicates the acidic nature of one of the components. As a result, these two metabolites were easily separated by first treating the mixture with excess of ethereal diazomethane to convert the acid compound to its methyl ester, followed by column chromatography. The methylated material was shown to be a mixture of esters of the fatty acids **3**, **4** and **5** by ^1H NMR spectroscopy and GC-mass spectroscopy.

The most polar material is a white crystalline compound (mp 167-168°C, Rf. 0.2 in 2% MeOH in $CHCl_3$) of molecular weight 428 ($C_{28}H_{44}O_3$).

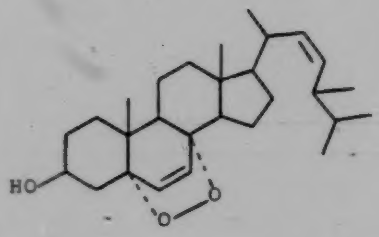
The HREIMS of this compound shows the loss of two oxygen atoms from the molecular ion as the most abundant fragment, a fragmentation characteristic of organic endoperoxides. The remaining fragment (after the loss of O_2) has the same molecular formula as ergosterol. This suggests that the compound may be ergosterol peroxide or an isomer of it.

The FTIR spectra of this compound shows the presence of a hydroxyl group at 3500 and 3350 cm^{-1} (free and associated OH).

The ^1H NMR of the compound agrees with the ^1H NMR data for ergosterol peroxide; δ 6.50 (1H, d), 6.24 (1H, d), 5.92 (1H, dd), 5.14 (1H, d), 3.96 (1H, ddd).

The ^{13}C NMR results for this peroxide show 28 carbons, as expected. Four carbons are sp^2-hybridized; δ 135.5 (d), 135.3 (d), 132.4 (d) and 130.8 (d), and form part of two disubstituted carbon-carbon double bonds. Three mono-oxygenated sp^3-carbons are detected; δ 82.2 (s), 79.5 (s) (attached to the two endoperoxide oxygens) and δ 66.5 (d) (attached to the hydroxyl group). The other ^{13}C NMR signals correspond to two tetrasubstituted carbons (singlets), six methine carbons (doublets), seven methylenes (triplets) and six methyls (quartets). The ^{13}C NMR signal multiplicity indicates forty three hydrogens directly attached to carbon, therefore only one hydroxyl group is present in the molecule.

Finally, comparison of this peroxide with an authentic sample of ergosterol peroxide shows the two to be identical.



Ergosterol peroxide

Other compounds present in the extracts of *Lachnellula fuscosanguinea* when grown in the fermentor are found in very small amounts, and since these extracts do not show any biological acitivity (as tested against *C. ulmi*, *S. aureus*, *C. albicans* and *E. coli*) further work on this extract was abandoned.

The bioassays for the crude extracts and active pure metabolites from L. Fuscosanguinea are described in Chapter 2 (see page 163).

Experimental

The distilled water used during the fungal culturing process was redistilled using an all glass apparatus. Fermentations were carried out in a New Brunswick Scientific MF-214 microferm laboratory fermentor or a magnaferm fermentor.

The solvents used for metabolite extraction and chromatographic separations were all distilled prior to use. Chloroform was recycled when used pure or with methanol; the concentration of methanol was measured by refractive index techniques using an ERMA refractometer.

Gas chromatographic (GC) analysis were carried out on a Hewlett-Packard 5700A gas chromatograph, using an APIEZON L analytical column and nitrogen as carrier gas. High pressure liquid chromatographic (HPLC) analysis were obtained on a Waters HPLC system. For centrifugal liquid chromatography (CLC) a Hitachi-Servo CLC-3 centrifugal liquid chromatograph, packed with Terochem thin layer chromatography silica gel G, was used. For column chromatography E. Merck silica gel 60 (finer than 230 mesh) was employed. E. Merck silica gel 60 (230-400 mesh ASTM) was used for flash chromatography. Terochem silica gel G containing 1% of Retma P-1 electronic phosphor from General Electric was utilized for thin layer preparative work

(20 x 20 cm plates, 0.35-0.50 mm layer thickness). Thin layer chromatographic analysis (TLC) were done on BDH precoated TLC plates of silica gel 60 F-254 (layer thickness 0.25 mm). The chromatograms were examined under ultraviolet light (254, 350 mm) or developed with iodine vapors. For analytical TLC, the visualization of the chromatograms was completed by spraying with 10% sulfuric acid, or with a solution of 5% phosphomolibdic acid containing a trace of ceric sulfate in 5% sulfuric acid, followed by careful charring on a hot plate.

Melting points are uncorrected and were determined on a Fisher-Johns melting point apparatus.

Refractive index values were recorded on an ERMA refractometer.

High resolution electron impact mass spectra (HREIMS) were recorded on an AEI MS-50 mass spectrometer. Chemical ionization mass spectra (CIMS) and low resolution electron impact mass spectra (LREIMS) were obtained using an AEI MS-4 mass spectrometer. Fast atom bombardment (FAB) spectra were obtained using a MS-50 spectrometer. The data were processed in DS-50 and DS-9 computers and reported as m/z (relative intensity).

Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 7199 FT interferometer. Single scan infrared (IR) spectra were recorded on a Perkin-Elmer 297 infrared spectrometer.

Ultraviolet (U.V.) spectra were obtained on a Unicam SP 1700 ultraviolet spectrophotometer, optical rotations on a Perkin-Elmer 141 polarimeter, and optical rotatory dispersion (ORD) curves on a Durrum Jasco ORD/UV-5 (SS-20 modification) recording spectropolarimeter. Routine ^1H nuclear magnetic resonance spectra were obtained on a Varian A-56/60A analytical spectrometer, a Bruker WP-80 spectrometer and a Varian HA-100 spectrometer with a Digilab FTS/NMR-3 data system. High field ^1H NMR and ^{13}C NMR spectra were recorded on Bruker WH-200 or WH-400 spectrometers with an Aspect 2000 computer system.

Cultivation of *Lachnellula fuscosanguinea*:

The strain C-693 (Canadian Forestry Service, accession number of the Commonwealth Mycological Institute IMI 250255 and 250256) of *Lachnellula fuscosanguinea* was found growing on *Pinus contorta* in Blue River, British Columbia in 1979. A sample of this fungus was obtained the same year from the Northern Alberta Forestry Research Center. The original slant tube culture was transferred to potato dextrose agar (PDA) slant tubes which were kept as stock cultures at 4°C.

Shake cultures of the fungi were obtained by transferring small pieces of mycelium from the slant tubes to 300 mL Erlenmeyer flasks containing 150 mL of sterile potato dextrose broth (PDB). After two weeks at 17°C the shake cultures were ready to use as inoculum.

When a fermentor was used, 450 mL of inoculum were transferred to 10 liters of sterile PDB in the fermentation tank. The variable conditions in the fermentor were kept as follows; temperature 20°C, air flow 1 liter/min, pressure 4 psi, drive speed 170 rpm and foam braker speed 600 rpm. The fermentation was worked up after three weeks.

In the case of still culture, 25 mL aliquots of inoculum were transferred to Fernbach flasks, each containing 1 liter of sterile PDB. The flasks were then kept at 17 to 20°C for at least 70 days. During a growth study two or three Fernbach flask cultures were harvested after 20, 27, 47, 77 and 110 days.

Extraction of the metabolites

The culture mixture was separated into broth and mycelium by filtration through cheese cloth. The broth was extracted, without previous concentration, by CH_2Cl_2 liquid-liquid extraction (3 x 500 mL of CH_2Cl_2 for every 3 liters of broth). The CH_2Cl_2 extract was then dried over anhydrous Na_2SO_4, filtered and concentrated to dryness under reduced pressure at 35°C. Higher yields of extract were obtained when the broth was first concentrated (5 liter to 500 mL, reduced pressure) and then extracted with ethyl acetate in a continuous liquid-liquid extractor.

The mycelium was air dried for two days, then extracted with CH_2Cl_2 in a Soxhlet extractor. The CH_2Cl_2 extract was dried (anhydrous Na_2SO_4) and concentrated. The crude extract yields from still culture, from fermentation, as well as the results for the growth study are summarized in Table II (see page 7).

Preliminary separation of the metabolites:

A. By Flash Chromatography

A 50 mm diameter column was filled with 8 inches of silica gel for flash chromatography, and packed using pure $CHCl_3$ as eluant. The sample (1.5 g of crude extract) was applied as a concentrated solution in chloroform and the column was eluted with solvent at the rate of 2 inches per minute while collecting 50 mL fractions. The polarity of the solvent was gradually increased by addition of methanol as indicated in Table IX.

Table IX. Polarity gradient of elution of a flash column

Solvent	Volume (mL)
$CHCl_3$	1150
1% MeOH in $CHCl_3$	600
3% MeOH in $CHCl_3$	500
5% MeOH in $CHCl_3$	1000
10% MeOH in $CHCl_3$	250
MeOH	500

The polarity and volume of the solvents used was decided on the basis of the visual development of the column, i.e., whenever a band appeared it was eluted without changing solvent polarity. Some bands were detected by ultraviolet light using a short wave - long wave U.V. lamp in the dark. The fractions obtained in this way were monitored by TLC and combined accordingly to give 10 to 15 final fractions. These fractions were subjected to further purification depending on the complexity and crystallinity of the mixtures.

B. By centrifugal liquid chromatography (CLC).

A 10 mm thick dish was prepared by pouring, in small portions, a slurry of 120 g of silica gel G for TLC in 500 mL of chloroform, into the CLC system rotating at 200 rpm. After each addition of the slurry the rotation speed was increased to 600 rpm to allow compact packing of the silica. Three grams of crude mycelium extract was applied to the disk and eluted as indicated in Table X.

Table X. Usual elution of a 10 mm CLC disk

Fractions	Tubes	Solvent	Volume (mL)
A, B, C	1-15	$CHCl_3$	1500
D, E, F, G	16-36	1% MeOH in $CHCl_3$	1000
G, H, I	37-47	5% MeOH in $CHCl_3$	700
I, J, K	48-65	10% MeOH in $CHCl_3$	1000

The fraction and elution pattern were determined on the basis of monitoring by U.V. chart detector and TLC. The fractions obtained in this manner were subjected to further purification as required.

PURIFICATION OF THE METABOLITES

Isolation of the mixture of triglycerides:

Fraction A from the CLC (242 mg) was applied to a flash chromatography column (5 cm) and eluted with Skellysolve B. The polarity of the solvent was increased by adding ethyl ether in ratios 2:1, 1:1 and 1:2 to the Skellysolve B. Six fractions were collected. Fraction A-2 (115 mg) contained the major compound present in the original mixture.

Column chromatography of fraction A-2 (30 g of silica gel G, mesh 70-270, 2.5 cm diameter column, CH_2Cl_2) gave eight fractions. Fraction A-2-b (61 mg) proved to be the pure triglycerides which were isolated as a pale yellow oil.
TLC: Rf 0.58 ($CHCl_3$)
FTIR ($CHCl_3$, cast): 3010, 2930, 2860, 1741, 1650, 1600, 1470, 1170 and 730 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$): δ 5.38 (9H, m, coupled to δ 4.25, 2.80 and 2.04), 4.25 (4H, ABX 2 x dd (4.0, 6.0 and 12.0 Hz), coupled to δ 5.38), 2.80 (1H, bt (5.0 Hz), coupled to δ 5.38), 2.34 (6H, t (7.0 Hz)), 2.04(8H, bt (4.0 Hz), coupled to δ 5.38), 1.62 (6H, bt (7.0 Hz)), 1.28 (51H, bs) and 0.90 (9H, vt).

HREIMS: m/z (formula, intensity, fragment) 602 ($C_{39}H_{70}O_4$, 17, M-R_1CO_2), 600 ($C_{39}H_{68}O_4$, 10, M-R_2CO_2), 577 ($C_{37}H_{69}O_4$, 75, M-R_3CO_2), 339 ($C_{21}H_{39}O_3$, 33, R_3 + 74), 336 ($C_{21}H_{36}O_3$, 11, R_2 + 74), 313 ($C_{19}H_{37}O_3$, 28, R_1 + 74), 264 ($C_{18}H_{32}O$, 51, R_3CO), 262 ($C_{18}H_{30}O$, 92, R_2CO), 239 ($C_{16}H_{31}O$, 19, R_1CO) and 81 (C_6H_9, 100.00).

CIMS: Maximum peak m/z 836 (7.4).

Transesterification of the mixture of triglycerides:

Triglycerides (114 mg) were dissolved in methanol (4 mL) and boron trifluoride etherate (2 mL). The mixture was stirred and heated at room temperature. After 1 h the reaction was stopped by adding water (50 mL) and extracting the resulting mixture with ether (3 x 20 mL). The combined ether extracts were dried over anhydrous Na_2SO_4 and concentrated to afford a yellow oil which appears as a single spot in a TLC analysis; Rf = 0.53 ($CHCl_3$:Skellysolve-B, ^1H NMR (80 MHz, $CDCl_3$): δ 5.40 (t), 3.70 (s), 2.80 (m), 2.3 (m), 2.05 (m), 1.65 (m), 1.3 (m) and 0.90 (vt). This mixture of methyl esters was dissolved in ether (10% solution), and 10 μl portions were subjected to gas chromatography - mass spectrometry (GC-MS) analysis using an Apiezon L (10%) column. Three major peaks were detected; m/z 270, 296 and 294.

Isolation of the C-24 fatty acid:

Fraction E from the CLC (41 mg) was dissolved in ether and treated with an excess of ethereal diazomethane solution. Evaporation of the solvent left an oily residue. Column chromatography of this residue (silica gel G, 2.5 cm diameter column), using as eluant a mixture of Skellysolve B and chloroform in a 1:1 ratio, gave in fractions E-2 to E-4 the pure methyl ester of the fatty acid as a waxy solid.

TLC: Rf 0.56 (Skellysolve B:chloroform 1:1).

FTIR ($CHCl_3$, cast): 2920, 2850, 1740, 1460, 1435, 1370, 1175, 1020, 885 and 730 cm^{-1}.

^1H NMR (100 MHz, $CDCl_3$): δ 3.69 (3H, s, OCH_3), 2.33 (2H, t (7.0 Hz), $-COCH_2-$), 1.56 (2H, bs, $-COCH_2CH_2-$), 1.27 (40H, bs, $-(CH_2)n$) and 0.90 (3H, vt (6.0 Hz), $-CH_3$).

HREIMS: m/z (formula, intensity, fragment) 382 ($C_{25}H_{50}O_2$, 28, M^+), 354 ($C_{23}H_{46}O_2$, 33, $M-C_2H_4$), 351 ($C_{24}H_{47}O$, 1.1, $M-OCH_3$), 326 ($C_{21}H_{42}O_2$, 20, $M-C_4H_8$), 298 ($C_{19}H_{38}O_2$, 59, $M-C_6H_{12}$) and 74 ($C_3H_6O_2$, 100, McLafferty fragment).

Isolation of a mixture of fatty acids, ergosterol peroxide and the orange dye:

The crude mycelium extract from fermentation (2.3604 grams) was separated into different fractions by flash chromatography (50 mm diameter column, $CHCl_3$). First, 27 x 50 mL fractions were collected, followed by 8 x 100 mL fractions.

Fractions 6 to 12 (900 mg) contained mostly the mixture of triglycerides and fractions 14 to 28 contained a small amount of a complex mixture composed mainly of the triglyceride and the same compounds present in fractions 29 to 35.

This last fraction (297.6 mg) was separated by partition chromatography using Sephadex LH-20 (50 g) and methanol (5 mL fractions). Fractions 18 to 29 contained a single compound, a crystalline orange dye (2.2 mg) soluble in methanol, acetone and ether, but only slightly soluble in chloroform.

TLC: Rf 0.22 (2% MeOH in $CHCl_3$).

Fractions 8-10 (246.4 mg) contained a mixture of two major compounds. One of these compounds was acidic as indicated by its behaviour in TLC studies. Treatment of the mixture with an excess of ethereal diazomethane gave two well separted spots (TLC) one of which corresponded to one of the compounds in the original mixture and the other to the methyl ester of the acidic material. This mixture was easily separated by column chromatography (silica gel G, $CHCl_3$) to give the two pure materials.

The least polar component (a mixture of esters of fatty acids) is a colorless oil (51 mg).

TLC: Rf 0.93 (2% MeOH in $CHCl_3$). Rf 0.43 (50% Skellysolve B in $CHCl_3$).

FTIR ($CHCl_3$, cast): 1740 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 5.35 (1H, m), 3.68 (3H, s), 2.80 (0.5 H, t), 2.34 (2H, t), 2.06 (1H, t), 1.65 (2H, bt), 1.3 (24H, bs), and 0.90 (3H, vt).

HREIMS: m/z (intensity): 298 (18.2), 294 (4.1), 270 (44.9), 296 (1.1), 264 (3.0).

The most polar component is a solid which was recrystallized from 50% Skellysolve B in ether to give a white crystalline compound (24.6 mg) mp 167-168°C.

TLC: Rf 0.20 (2% MeOH in $CHCl_3$).

FTIR ($CHCl_3$, cast): 3500, 3350, 2950, 2875, 2360, 2330, 2465, 2380, 2050 and 975 cm^{-1}.

HREIMS: m/z (formula, intensity, fragment): 428 ($C_{28}H_{44}O_3$, 9.4, M^+), 413 ($C_{27}H_{41}O_3$, 4.9, $M-CH_3$), 410 (12.6, $M-H_2O$), 396 ($C_{28}H_{44}O$, 100.0, $M-O_2$), 303 ($C_{19}H_{27}O_3$, 6.7, $M-C_9H_{17}$). ^1H NMR (400 MHz, $CDCl_3$): δ 6.50 (1H, d, 8.0 Hz), 6.24 (1H, d, 8.0 Hz), 5.22 (1H, dd, 7.5 and 14.0 Hz), 5.14 (1H, dd, 7.0

and 14.0 Hz), 3.96 (1H, ddd, 5.0, 11.0 and 16.0 Hz), 2.10 (1H, ddd, 2.0, 5.0, 12.0 Hz), 2.01 (1H, m), 1.91 (2H, m), 1.85 (2H, m), 1.72 (3H, m), 1.56 (1H, m), 1.50 (3H, m), 1.38 (1H, m), 1.24 (4H, m), 1.00 (3H, d, 62 Hz), 0.91 (3H, d, 6.5 Hz), 0.89 (3H, s), 0.83 (3H, d, 6.5 Hz), 0.82 (3H, s), 0.82 (3H, d, 6.5 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 135.50 (d, 28), 135.17 (d, 31), 132.43 (d, 24), 130.80 (d, 25) 82.19 (s, 12), 79.45 (s, 13), 66.48 (d, 39), 56.35 (d, 25), 51.78 (d, 29), 51.27 (t, 27), 44.64 (s, 22), 42.85 (d, 27), 39.68 (d, 30), 39.46 (t, 23), 37.05 (t, 38), 34.80 (t, 25), 33.17 (d + t, 28), 30.21 (t, 25), 28.62 (d, 24), 23.47 (s, 22), 20.92 (q, 23), 20.69 (q, 23), 19.95 (t, 19), 19.66 (q, 20), 18.19 (q, 21), 17.59 (q, 21), 12.93 (q, 21).

Isolation of the nitrogen containing compounds.

Fraction M (73.6 mg) obtained from the flash chromatography column of the crude mycelium extract eluted with 10% methanol in $CHCl_3$, was triturated with CH_2Cl_2 to give a CH_2Cl_2 insoluble white solid. This solid was recrystallized four times from hot methanol to give 5.8 mg of small white crystals. Mp 140-142°C.

TLC: Rf 0.58 (2% MeOH in $CHCl_3$).

FTIR ($CHCl_3$, cast): 3340, 3200, 2920, 2860, 1740, 1620, 1545, 1465, 1165 and 720 cm^{-1}.

HREIMS: m/z (formula, intensity, fragment) 683 ($C_{42}H_{85}NO_5$, 1.58, M^+), 665 ($C_{42}H_{83}NO_4$, 39, M-H_2O), 647 ($C_{42}H_{81}NO_3$, 60, M-$2H_2O$), 439 ($C_{27}H_{53}NO_3$, 35, M-$C_{15}H_{32}O_2$), 409 ($C_{26}H_{51}NO_2$, 33, M-$C_{16}H_{34}O_3$), 399 ($C_{25}H_{46}O_3$, 28, M-$C_{17}H_{39}NO_2$), 384 ($C_{24}H_{50}NO_2$), 72, M-$C_{18}H_{35}O_3$), 357 ($C_{20}H_{39}NO_4$, 82, M-$C_{22}H_{46}O$), 339 ($C_{20}H_{37}NO_3$, 100.00, M-$C_{22}H_{48}O_2$), 60 ($C_2H_6NO_2$, 73). ^{1}H NMR (400 MHz, pyridine-$\underline{d}_5$): δ 8.60 (1H, d, J = 8.0 Hz), 6.72 (2H, bs), 6.23 (1H, bs), 5.14 (1H, sextet, J =4.5 Hz), 4.65 (1H, bd, J = 4.0 Hz), 4.55 (1H, dd, J = 4.5 Hz), 4.45 (1H, dd, J = 4.5 Hz), 4.39 (1H, bt, J = 5.0 Hz), 4.31 (1H, bt, J = 6.0 Hz), 2.28 (2H, m), 2.08 (1H, m), 1.96 (2H, m), 1.79 (4H, m), 1.42-1.31 (70H, m), 0.90 (6H, vt).

Isolation of Lachnellulone:

Fraction E (176.3 mg) from flash chromatography of the crude mycelium extract was dissolved in hot Skellysolve B and slowly cooled to room temperature. The mother liquors were separated from the crystals by suction using a micropipette and the recrystallization was repeated once more to afford 20 mg of white crystals (mp = 126-127°C).

TLC: Rf 0.44 ($CHCl_3$).

FTIR ($CHCl_3$, cast): 3400 (OH), 2950, 2925, 2860, 1708 (C=O), 1670 (C=C-C=O), 1560, 1460, 1380, 1305, 1090 and 600 cm^{-1}. UV (MeOH) λ_{max} 218 (ε = 8670), 275 (ε 16200).

$[\alpha]_D^{25}$ + 48.4 (C10, CH_3OH)
HREIMS: m/z (formula, intensity): 324 ($C_{18}H_{28}O_5$, 1.30), 308 ($C_{18}H_{28}O_4$, 256), 239 ($C_{13}H_{19}O_4$, 3.86), 226 ($C_{12}H_{18}O_4$, 2.10), 224 ($C_{12}H_{16}O_4$, 10.12), 211 ($C_{11}H_{15}O_4$, 100.00), 193 ($C_{11}H_{13}O_3$, 45.97). ^1H NMR (400 MHz, $CDCl_3$): δ 5.02 (1H, quintet; J = 7.0 Hz, coupled to 2.33, 1.87, 1.71), 4.57 (1H, ddd, J = 3.0, 6.5 and 10.0 Hz, coupled to 4.45, 1.71 and 1.30), 4.45 (1H, dd, J = 3.0, 6.5 Hz, coupled to 4.57 and 3.92), 3.92 (1H, d, J = 3.0 Hz, coupled to 4.95), 3.76 (1H, ddd, J = 6.0, 10.0, 21.0 Hz, coupled to 3.32, 2.33, 1.87), 3.32 (1H, ddd, J = 7.0, 10.0, 20.0 Hz, coupled to 3.76, 2.33 and 1.87), 2.33 (1H, dddd, J = 6.0, 7.0, 10.0, 13.0 Hz, coupled to 5.02, 3.76, 3.32 and 1.87), 1.87 (1H, dddd, J = 7.0, 7.0, 10.0 and 12.0), 1.71 (2H, m, coupled to 5.02, 4.57, 1.87, 1.42 and 1.30), 1.60 (1H, m), 1.42 (4H, m, coupled to 1.71 and 0.96), 1.30 (6H, m, coupled to 4.57, 1.70 and 0.89), 0.96 (3H, t, J = 7.0 Hz, coupled to 1.92), 0.89 (3H, vt, J = 7.0 Hz, coupled to 1.30). ^{13}C NMR (100.6 MHz, CD_2Cl_2): δ 191.1 (s, 10), 189.0 (s, 15), 165.3 (s, 10), 100.2 (s, 7), 91.4 (d, 43), 77.6 (d, 46), 71.9 (d, 47), 36.4 (t, 95), 34.9 (t, 48), 31.9 (t, 65), 28.7 (t, 37), 27.9 (t, 55), 27.2 (t, 45), 25.9 (t, 69), 22.9 (t, 69), 22.8 (t, 66), 14.1 (q, 58), 14.0 (q, 57).

Deuterium-labelled Lachnellulone:

A few milligrams of lachnellulone was dissolved in CH_2Cl_2 and mixed with two drops of D_2O. After shaking the mixture vigorously, the solvent was removed by evaporation. The labelled lachnellulone shows a ^1H NMR spectra corresponding to that of unlabelled lachnellulone but with changes in two signals. ^1H NMR (400 MHz, $CDCl_3$) δ 3.92 (absent) δ 4.41 (d, $J = 6.5$ Hz).

HREIMS: m/z (formula, intensity) 325 ($C_{18}H_{27}DO_5$, 1.36), 324 ($C_{18}H_{28}O_5$, 1.86), 211 ($C_{11}H_{15}O_4$, 100.00), 212 ($C_{11}H_{14}DO_4$, 60).

p-Nitrophenylhydrazone of Lachnellulone:

Lachnellulone (4.0 mg, 1.2×10^{-2} mmoles) was dissolved in 95% ethanol (0.5 mL) and treated with *p*-nitrophenylhydrazine (4.0 mg, 2.5×10^{-2} mmoles). The solution was warmed to 80°C in a water bath and one drop of glacial HOAc was added. The resulting solution was kept at 80°C for 20 minutes. After slowly cooling to room temperature the solvent was evaporated under vacuum. The residue was dissolved in CH_2Cl_2 and allowed to evaporate to dryness. The crystals of *p*-nitrophenylhydrazone were washed with ether and the ether solution evaporated to dryness. This residue was purified by prep. thin layer chromatography (20 x 20 cm plate, 5% MeOH in $CHCl_3$, 0.35 mm layer thickness, triple elution).

The p-nitrophenylhydrazone was obtained as a semicrystalline yellow material (3.0 mg).

TLC: Rf 0.30 (5% MeOH in $CHCl_3$).

HREIMS: m/z (formula, intensity, fragment). 441 ($C_{24}H_{31}N_3O_5$, 2.37, M-H_2O), 402 ($C_{20}H_{24}N_3O_6$, 81.45, M-C_4H_7), 389 ($C_{20}H_{22}N_3O_5$, 13.26, M-C_4H_7-H_2O), 373 ($C_{19}H_{23}N_3O_5$, 39.22, M-$C_5H_{10}O$), 359 ($C_{18}H_{21}N_3O_5$, 53.02, M-$C_6H_{12}O$), 273 ($C_{13}H_{11}N_3O_4$, 100.00, M-$C_5H_{10}O$-$C_6H_{12}O$), 253 ($C_{12}H_{17}N_2O_4$, 5.49, M-$C_{12}H_{16}NO_2$), 235 ($C_{12}H_{15}N_2O_3$, 12.05, M-$C_{12}H_{16}NO_2$-H_2O). ^1H NMR (400 MHz, $CDCl_3$): δ 8.33 (2H, d, J = 9 Hz), 8.02 (2H, d, J = 9 Hz), 4.52 (1H, bd, J = 10 Hz), 4.40 (1H, dd, J = 60 Hz), 4.29 (1H, bd, J = 10.0 Hz), 3.69 (1H, bm), 2.96 (2H, m), 2.80 (1H, b), 2.08 (1H, m), 1.88 (2H, m), 1.75 (1H, m), 1.60 (1H, m), 1.3 to 1.4 (~ 13H, m), 0.92 (3H, t), 0.90 (3H, t). ^1H NMR (400 MHz, $CDCl_3$ + D_2O): δ 4.52 (1H, d, J = 1.5 Hz), δ 4.24 (disappears), δ 2.80 (disappears).

Lachnellulone acetate:

Lachnellulone (3.0 mg, 9.2×10^{-3} mmoles) was dissolved in CH_2Cl_2 (0.5 mL). Acetyl chloride (0.5 mL) was added, with constant stirring, to the lachnellulone solution. After 2 h at room temperature a drop of dry pyridine was added and the solution was then stirred for 24 h at room temperature. The reaction was worked up by adding water (2 mL) and chloroform (1 mL) and vigorously shaking the two phase system. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness to give Lachnellulone acetate (3.0 mg).

TLC: Rf 0.30 (1% MeOH in $CHCl_3$).

HREIMS: m/z (formula, intensity, fragment) 366 ($C_{20}H_{20}O_6$, 2.26, M^+), 253 ($C_{13}H_{17}O_5$, 21.34, M-[illegible]), 211 ($C_{11}H_{15}O_4$, 100.00, M-$C_9H_{15}O_2$), 193 ($C_{11}H_{13}O_3$, 16.85, M-$C_9H_{17}O_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 5.53 and 5.48 (1H, 2 x d, J = 4.5 Hz), 4.92 (1H, m), 4.44 (1H, m), 3.65 (1H, m), 3.32 (1H, m), 2.30 (1H, m), 2.21 and 2.19 (1H, 2 x s), 1.89 (1H, m), 1.64 (2H, m), 1.40-1.31 (14H, bm), 0.94 (3H, t), 0.90 (3H, t).

Lachnellulone *p*-nitrophenylhydrazone-diacetate:

Lachnellulone *p*-nitrophenylhydrazone (3.0 mg, 6.5 x 10^{-3} mm) was dissolved in dry pyridine (0.3 mL). This solution was stirred and three drops of acetic anhydride was added. The reaction was then stirred at room temperature for 48 h. After this time the pyridine was azeotropically removed with toluene. The dark residue remaining was passed through a 10 mm diameter flash column using chloroform as eluant to give in fractions 4 and 5 3.5 mg (100% yield) a yellow semi-solid material that corresponds to Lachnellulone p-nitrophenylhydrazone diacetate.

TLC: Rf 0.45 ($CHCl_3$)

HREIMS: m/z (formula, intensity) 543 ($C_{28}H_{37}N_3O_8$, 13%) 483 ($C_{26}H_{33}N_3O_4$, 69), 424 ($C_{24}H_{30}N_3O_4$, 100%), 414 ($C_{21}H_{24}N_3O_4$, 20), 401 ($C_{20}H_{23}N_3O_6$, 70%) and 380 ($C_{21}H_{22}N_3O_4$, 30%). ^1H NMR (200 MHz, $CDCl_3$): δ 8.33 (2H, d, J = 9 Hz), 7.61 (2H, d, J = 9.0 Hz), 6.16 (1H, d, J = 2.0 Hz, coupled to δ 4.52), 4.90 (1H, quintet, J = 6.0, coupled to δ 2.09), 4.52 (1H, ddd, J = 2.0, 4.0 and 9.0 Hz, coupled to δ 6.16 and 1.81), 3.01 (2H, m, coupled to δ 2.09), 2.13 (3H, s), 2.09 (2H, m, coupled to δ 4.90 and 3.09), 2.06 (3H, s), 1.81 (1H, m), 1.58 (5H, m), 1.30 (8H, m) and 0.90 (6H, vt).

Synthesis of ethyl 2-ethoxy-4,5-dihydro-4-oxofuran-3-carboxylate (**13**).

Magnesium metal (0.207 atg) was placed in a two neck 250 mL round bottom flask fitted with a reflux condenser with a drying tube and a dropping funnel. The system was flushed with dry nitrogen, and dry ethanol (15 mL) and carbon tetrachloride (1.0 mL) were added to the magnesium to give a slow but steady formation of hydrogen. After 20 minutes more dry ethanol (21 mL) was added slowly through the funnel, and the mixture was heated to reflux for 1.5 h.

Freshly distilled diethyl malonate (33.7 g) was added dropwise and with constant stirring to the reaction mixture. The mixture reacted vigorously and all the magnesium was dissolved after heating under reflux for 1.5 h.

Dry ether (100 mL) was added dropwise to the resulting green solution and the mixture was heated to reflux for four hours. The reaction mixture was then cooled in an ice-water bath and chloroacetyl chloride (16 mL) was added slowly. The resulting thick green suspension was heated to reflux for one hour to produce a color change to yellow. This yellow solution was cooled to 0°C and treated with a mixture of 3N H_2SO_4 and ice (~ 100 mL). The two resulting layers were separated in a separatory funnel and the aqueous layer was washed twice with ether. The ethereal extracts were combined and worked up in the usual way to give a clear yellow oil which was the uncyclized form of the desired product. Cyclization was accomplished by dissolving the yellow oil in dry benzene (450 mL) containing dry triethylamine (55 mL). The deep yellow solution was heated to reflux for 30 min, and after cooling for 2 hours a precipitate was formed. Filtration gave a clear yellow solution which after concentration afforded a brown solid residue. Recrystallization of the residue from carbon tetrachloride yielded the desired product as pale yellow needles (mp 90-91°C).

IR($CHCl_3$, cast): 2985, 2960, 2940, 2890, 1700, 1590, 1485, 1390, 1356, 1240, 1095, 1000, 840, 780 and 720 cm^{-1}.

UV (CH_3OH): λ_{max} 222 (t, 12500), 248 (ε 17800).

^1H NMR (80 MHz, $CDCl_3$): δ 4.70 (2H, s), 4.60 (2H, q), 4.20 (2H, q), 1.48 (3H, t) and 1.20 (3H, t).

^{13}C NMR (100 MHz, $CDCl_3$): δ 190.7, 183.8, 161.4, 108.9, 75.4, 67.9, 59.9, 14.5, 14.4. This compound is unstable and must be stored at 0°C.

Determination of gated decoupled ^{13}C spectra:

A concentrated sample of the compound under study was prepared in the usual way for NMR studies. The concentration of the sample should be about 0.5 molar or higher to obtain good practical results and to decrease the collection time required. However, more dilute samples, with concentrations as for normal PND ^{13}C NMR studies can be used if enough scans are collected. Using the aspect 2000 microcomputer the following program is activated:

1 - ZE
2 - BB
3 - D1
4 - DO
5 - D2

6 - GO = 2

7 - Exit

The time delays D1 and D2 are set as 0.5 and 5.0 milliseconds, respectively. The O2 value is set in the proton region as for PND ^{13}C NMR and the decoupling power is usually set as 7 Debyes or 5 Debyes depending on whether the Bruker WH-200 or WH-400 is being used.

The number of scans varies depending on the concentration of the sample, but it usually ranges between 10,000 to 200,000 scans.

To obtain a clear picture of the long range coupling constants two data manipulation techniques are usually employed; zero filling and resolution enhancement.

The zero filling effect is obtained by increasing the data points from 16K to 32K (50 MHz) or from 32K to 64K (100 MHz).

The resolution enhancement is accomplished by the use of a negative line broadening (negative of the natural line broadening), usually between -1.5 and -3.0, and by multiplying the FID by a Gaussian Multiplier the enhancing properties of which are controlled by a parameter called Gaussian Broadening function (GB). This can be varied from 0 to 1 depending on the amount of enhancement needed and on the corresponding decrease of the signal to noise ratio.

C

The gated ^{13}C NMR of compound **13** was determined to be: δ 190 (t, J = 3.5 Hz), 182 (quintet, J = 3.2 Hz), 160 (t, J = 3.5 Hz), 88 (bs), 74 (t, J = 152.5 Hz), 67 (qt, J - 4.5, 149.5 Hz), 58 (qt, J = 4.5, 145 Hz), 13.1 (tq, J = 2.5, 129.5 Hz) and 13.0 (tq, J = 2.7, 120.5 Hz).

The gated ^{13}C NMR for compound **15** is: δ 181 (bd), 168 (dq), 161 (dq), 112 (bd), 89 (dq), 56 (q) and 19 (dq).

Rearrangement of Lachnellulone:

Lachnellulone (8 mg) was dissolved in methanol (1.0 mL), and the solution was made basic to pH 10 with 1 M NaOH. The solution was stirred for 3 h at room temperature. The resulting solution was acidified to pH 1 with 5% HCl and diluted with distilled water. Extraction of the aqueous solution with ethyl acetate afforded a yellow solid which was purified by flash chromatography (10 nm diameter column, 2% MeOH in $CHCl_3$). Isolachnellulone was obtained as a white solid (4.8 mg). Rf. 0.3 in 2% MeOH in $CHCl_3$.

HREIMS: m/z (formula), 324 ($C_{18}H_{28}O_5$), 306 ($C_{18}H_{26}O_4$, 253 ($C_{13}H_{17}O_5$), 224 ($C_{12}H_{16}O_9$, 100%), 206 ($C_{12}H_{14}O_3$), 195 ($C_{11}H_{15}O_3$), 195 ($C_{10}H_{11}O_4$), 193 ($C_{11}H_{13}O_3$), 181 ($C_9H_9O_4$), 154 ($C_7H_6O_4$), 142 ($C_6H_6O_4$) and 127 ($C_5H_3O_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (1H, m, coupled to δ 2.37, 1.95, 1.87 and 1.71), 4.42-4.38 (1H, 4 x d, coupled to δ 4.42-4.38, 2.70, 1.75 and 1.68), 3.59 (1H, m, coupled to δ 3.26, 2.37 and 1.87), 3.26 (1H, m, coupled to δ 3.59, 2.37 and 1.87), 2.70 (½ H, bdd, coupled to δ 3.86, exchanged with CD_3OD), 2.37 (1H, m, coupled to δ 4.94, 3.59, 3.26 and 1.87), 1.95 (1H, m, coupled to δ 4.94, 1.75 and 1.41), 1.87 (1H, m, coupled to 4.94, 3.59, 3.26 and 2.37), 1.75 (1H, m, coupled to δ 4.94, 4.02, 1.95, 1.50), 1.68-1.62 (2H, m, coupled to 4.02, 2.70, 1.95), 1.50 (1H, m, coupled to δ 1.95 and 1.75), 1.41 (4H, m), 1.32 (6H, m), 0.94 (3H, t) and 0.90 (3H, vt).

Acetylation of Isolachnellulone:

Isolachnellulone (4.5 mg) was dissolved in dry pyridine (0.5 mL) and treated with acetic anhydride (five drops). The colorless solution was stirred at room temperature for 10 h to give a dark brown solution that contains a complex mixture of at least four compounds as indicated by TLC.

Gated decoupled spectrum of Lachnellulone and selective decoupling experiments.

The gated decoupled spectrum of lachnellulone was determined using a sample of 50 mg of lachnellulone in 0.5 mL of $CDCl_3$, as explained for ethyl 2-ethoxy-4,5-dihydro-4-oxofuran-3-carboxylate.

The selective decoupling experiments were determined using this same sample of lachnellulone. First the ^1H NMR spectrum of lachnellulone was determined using the ^{13}C NMR prove in order to calibrate the different irradiation frequencies. The decoupling was obtained using the single frequency (cw) decoupling mode. The results as summarized in the following table.

Table XI. Gated and selective decoupling of lachnellulone.

δ (ppm)	multiplicity	J(Hz)	Decoupled at * δ 5	δ 4.5	δ 3.5
191	dt	10,2.5	bt	s	bd
188	m	-	m	m	t
164	d	8.0	bd	s	d
100	s	-	s	s	s
91	dm	152,-	m	dm	dm
76	dm	149,-	dm	m	dm
72	dd	149,-	dd	bs	dd
36	tm	50,-	tm	tm	m

*Decoupling at a given frequency always affected other signals in the neighborhood.

Dehydration of lachnellulone:

Lachnellulone (2 mg) was dissolved in dry pyridine (5 mL) and treated with $SOCl_2$ (2 drops) at 0°C. After stirring the mixture for 12 h TLC analysis showed extensive decomposition of the natural product.

The experiment was repeated using $POCl_3$ at 0°C, but after 12 h the starting material remained unchanged. When the reaction mixture was let to warm up to room temperature it rapidly became colored, and TLC analysis showed decomposition of the starting material.

Lachnellulone mesylate:

Lachnellulone (20 mg) was dissolved in CH_2Cl_2 (2 mL) and pyridine (10 drops), and treated with mesyl chloride (10 drops). The reaction mixture was stirred overnight at room temperature, and the resulting yellow solution was evaporated to dryness and dissolved in $CHCl_3$. This solution was washed with 5% HCl (2 x 5 mL), dried over anhydrous Na_2SO_4 and concentrated to give a single compound in quantitative yield.

TLC: Rf 0.44 in $CHCl_3$.

HREIMS: m/z (formula, intensity, fragment), 402 ($C_{19}H_{30}SO_7$, 0.6, M^+), 323 ($C_{18}H_{27}O_5$, 100, M-CH_3SO_2), 211 ($C_{11}H_{15}O_4$, 31.4, M-$C_8H_{15}SO_3$) and 193 ($C_{11}H_{13}O_3$, 85).

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.12 , 5.02 (1H, 2 x d, J = 3.5 Hz), 4.96 (1H, m), 4.48 (1H, m), 3.60 (1H, m), 3.40 (1H, m), 3.24 (3H, 5), 2.35 (1H, m), 1.9 (1H, m), 1.75 (3H, m), 1.4 (~ 12H, m) and 0.95 (6H, vt).

Decomposition of Lachnellulone mesylate:

Lachnellulone mesylate (2 mg) was dissolved in toluene (1 mL) with DBU (2 drops). The mixture was stirred for 12 h at 40°C. The reaction mixture was concentrated to dryness and the residue left in the flask was separated in a flash column (10 mm diameter column, benzene:acetone:HOAc 75:25:1). Fractions 7-9 from the flash column gave 0.9 mg of a material that has been provisionally identified as a rearranged product of lachnellulone (see page 68).

TLC: Rf 0.25 in benzene:acetone:HOAc 75:25:1.

^{1}H NMR (200 MHz, $CDCl_3$): δ 5.92 (1H, dt, J = 16, 7 Hz, coupled to δ 5.52, 2.11), 5.52 (1H, dd, J = 15, 8 Hz, coupled to δ 5.92, 4.48), 4.48 (1H, bd, J = 7 Hz, coupled to δ 5.52), 3.4-3.2 (8H, m), 2.55 (2H, bd, J = 10 Hz), 2.11 (2H, dd, J = 7 Hz, coupled to δ 5.92, 1.41), 1.7-1.3 (m) and 0.90 (6H, vt).

Isolation of compound T-1:

Flash chromatography (50 mm diameter column, $CHCl_3$ and 2% MeOH in $CHCl_3$) of a new crude still culture mycelium extract (2.0 g) gave in fractions 2 and 3 an oily residue which when treated with ether left an insoluble solid. After washing with ether three times the dust-like crystals of this material became white (mp 239-240°C).

FTIR (KBr pellet): 3420, 2950, 2920, 2845, 1660, 1590 (shoulder at 1610), 1419 and 1280 cm^{-1}.

UV (MeOH): λ_{max} 241 nm (ε 352), 273 nm (ε 365).

UV (MeOH + NaOH): λ_{max} 250 nm (ε 400), 270 nm (ε 375).

UV (MeOH + HCl): λ_{max} 219 nm (ε 242), 275 nm (ε 317).

^1H NMR (400 Mhz, DMSO-$\underline{d}_6$): δ 4.10 (1H, m), 3.39 (m, H_2O), 2.71 (1H, m), 2.31 (1H, m), 1.58 (1H, m), 1.45 (1H, m), 1.3-1.2 (16H, m), 0.90 (6H, 2 x vt).

^1H NMR (400 MHz, Pyridine-$\underline{d}_5$): δ 5.02 (7H, m), 4.27 (1H, bm, coupled to 3.26, 2.56, 1.64), 3.38 (1H, ddd, J = 6.0, 8.0, 15.0 Hz, coupled to 4.27, 2.56), 3.26 (1H, ddd, J = 6.0, 8.5, 15.0 Hz, coupled to 4.27, 2.56), 2.56 (2H, m, coupled to 4.27, 3.26 and 1.88), 1.88 (2H, m, coupled to 3.38, 3.26, 1.5-1.2), 1.64 (1H, m, coupled to 3.38, 3.26, 1.5-1.2), 1.5-1.2 (16H, m), 0.86 (3H, virtual triplet), 0.82 (3H, vt).

Isolation of Lachnelluloic Acid:

Fraction C (305 mg) from flash chromatography of the crude mycelium extract was a waxy crystalline subtance. Column chromatography of this fraction (40 g of silica gel G mesh 270, 2 cm diameter column, Skellysolve B: chloroform 1:1) gave a pure crystalline substance (204.2 mg) mp 39-40°C. $[\alpha]_D^{25}$ - 26.6° (c 10 MeOH).

TLC: Rf 0.5 (Skellysolve B: $CHCl_3$:HOAc:50:50:1).

FTIR ($CHCl_3$, cast or solution): 2955, 2925, 2860, 1708, 1695, 1555, 1465 and 1065 cm^{-1}.

UV (MeOH): λ_{max} 218 nm (ε 700), 274 nm (ε 1090).

UV (MeOH + NaOH): λ_{max} 250 nm (ε 1375).

UV (MeOH + HCl): λ_{max} 275 nm (ε 1095).

HREIMS: m/z (formula, intensity, fragment) 310 ($C_{18}H_{30}O_4$, 33, M^+), 292 ($C_{18}H_{28}O_3$, 6, M-H_2O), 239 ($C_{13}H_{19}O_4$, 100.0, M-C_5H_{11}) 221 ($C_{13}H_{17}O_3$, 62, M-$C_5H_{13}O$).

^1H NMR (400 MHz, $CDCl_3$): δ 4.38 (1H, dddd, J = 4.0, 5.0, 7.0 and 11.0 Hz, coupled to 2.58, 2.68, 1.80 and 1.60), 3.06 (1H, ddd, J = 6.5, 8.5 and 15.0 Hz, coupled to 1.66), 2.96 (1H, ddd, J = 6.5, 8.5, 15.0 Hz, coupled to 1.66), 2.68 (1H, dd, J = 11.0, 17.0 Hz, coupled to 4.38 and 2.58), 2.58 (1H, dd, J = 4.0, 17.0 Hz, coupled to 4.38 and 2.68), 1.80 (1H, dddd, J = 5.0, 7.0, 10.0, 13.0 Hz, coupled to 4.38, 1.66 and 1.53), 1.66 (3H, m, coupled to 4.38, 3.06, 2.96 and 1.80), 1.53 (1H, quintet, J = 5.0), 1.34-1.30 (14H, m, coupled to 0.90 and 0.92), 0.92 (3H, vt, J = 6.0, coupled to 1.34 and 1.30), 0.90 (3H, vt, J = 7.0, coupled to 1.34 and 1.30). ^{13}C NMR (100 MHz, $CDCl_3$): δ 209.22 (s, 17), 194.88 (s, 20), 163.92 (s, 13), 102.93 (s, 16), 73.57 (d, 3s), 38.25 (t, 33), 37.65 (t, 38), 34.40 (t, 38), 31.43 (t, 29), 31.22 (t, 32), 29.05 (t, 32), 28.75 (t, 26), 24.82 (t, 26), 24.14 (t, 34), 22.33 (t, 25), 22.21 (t, 29), 13.76 (q, 22) and 13.66 (q, 21). Positive ferric chloride test.

Reaction of Lachnelluloic acid with diazomethane:

Lachnelluloic acid (1.0 mg) was dissolved in ether and then treated with an excess of freshly prepared ethereal diazometate. The reaction was kept at room temperature for 10 min, with constant stirring. Evaporation of the solvent and the excess of diazomethane with a nitrogen stream left a yellowish residue which on TLC showed the presence of six components. (Skellysolve B:$CHCl_3$:HOAc:50:50:1). Repetition of this process gave exactly the same result.

Attempted Acetylation of Lachnelluloic Acid:

Lachnelluloic acid (1.7 mg) was dissolved in chloroform (0.5 mL) containing 3 drops of pyridine and 3 drops of acetic anhydride. The reaction mixture was stirred at room temperature for 12 h. No reaction was detected after this period of time. The reaction was repeated using DMAP as catalyst, but the outcome was the same. Lachnelluloic acid (10.0 mg) was dissolved in $CHCl_3$ (0.5 mL) and acetic anhydride (4 drops) and treated with concentrated H_2SO_4 (four drops). No reaction was detected after stirring the reaction mixture for 24 h at room temperature. Heating the same reaction mixture to 55°C for 24 h did not produce any acetylated product and practically all the starting material was recovered.

Hydrogenation of dehydroacetic acid (**7**):

Dehydroacetic acid (3.7 mmoles) was mixed with 10% Pd-C (0.22 g) and ethyl acetate (40 mL). The reaction mixture was pressurized to 60 psi with hydrogen and shaken at room temperature for 24 h. After filtration, the reaction mixture was concentrated in vacuo to afford a solid residue. Flash chromatography of this solid (40 mm diameter column, $CHCl_3$:Hexane:HOAc:100:50:3) afforded, in fractions 12 to 20, white crystals of dihydrodehydroacetic acid (**8**, 60% yield), mp. 88-89°C.

TLC: Rf 0.3 $CHCl_3$:Skellysolve B:HOAc:2:1:1%.

FTIR (film): 2980, 2920, 1715, 1560, 1458, 1080 and 770 cm^{-1}.

UV (MeOH): λ_{max} 219 (ε 20700), 274 (ε 26100) nm.

UV (MeOH + NaOH): λ_{max} 208, 248 nm.

^1H NMR (200 MHz, $CDCl_3$): δ 17.9 (1H, s), 4.52 (1H, m), 2.63 (2H, dd, J = 2.5, 6 Hz), 2.60 (3H, s), 1.44 (3H, d, J = 6 Hz).

^{13}C NMR (50 MHz, $CDCl_3$): δ 201.18 (s), 195.04 (s), 164.35 (s), 103.43 (s), 70.25 (d), 39.45 (t), 26.47 (q) and 20.56 (q).

The gated ^{13}C NMR spectra for dihydrodehydro lachnelluloic acid (**8**): δ 201 (qd, J = 6.5, 2.5 Hz), 195 (m), 164 (bs), 103 (m), 70 (dq, J = 146.0, 3.5 Hz), 39 (tq, J = 120.5, 5.0 Hz), 26 (qbd, J = 129.5, 1.5 Hz) and 20 (qq, J = 117.5, 3.0 Hz).

The gated ^{13}C NMR spectra for D-labelled dihydrodehydroacetic acid (**9**): δ 201 (q, J = 6.5 Hz), 194 (m), 164 (bs), 103 (m), 70 (ddm, J = 147.5, 3.5 Hz), 39 (tbq, J = 130.0, 4.5 Hz), 26 (q, J = 230 Hz) and 20 (qq, J = 127.5, 2.0 Hz).

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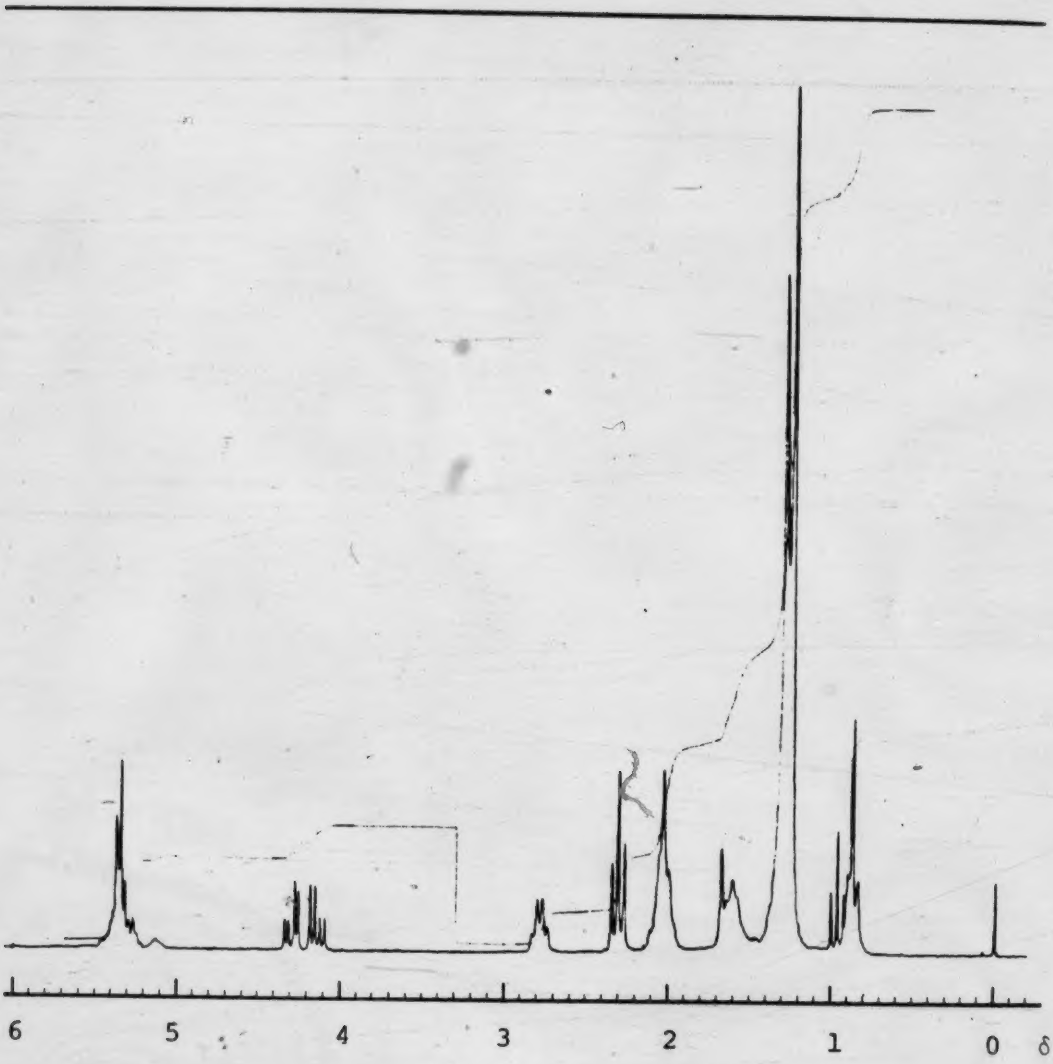


FIGURE 1: 200 MHz ^{1}H NMR spectrum (CDCl$_3$) of the mixture of triglycerides.

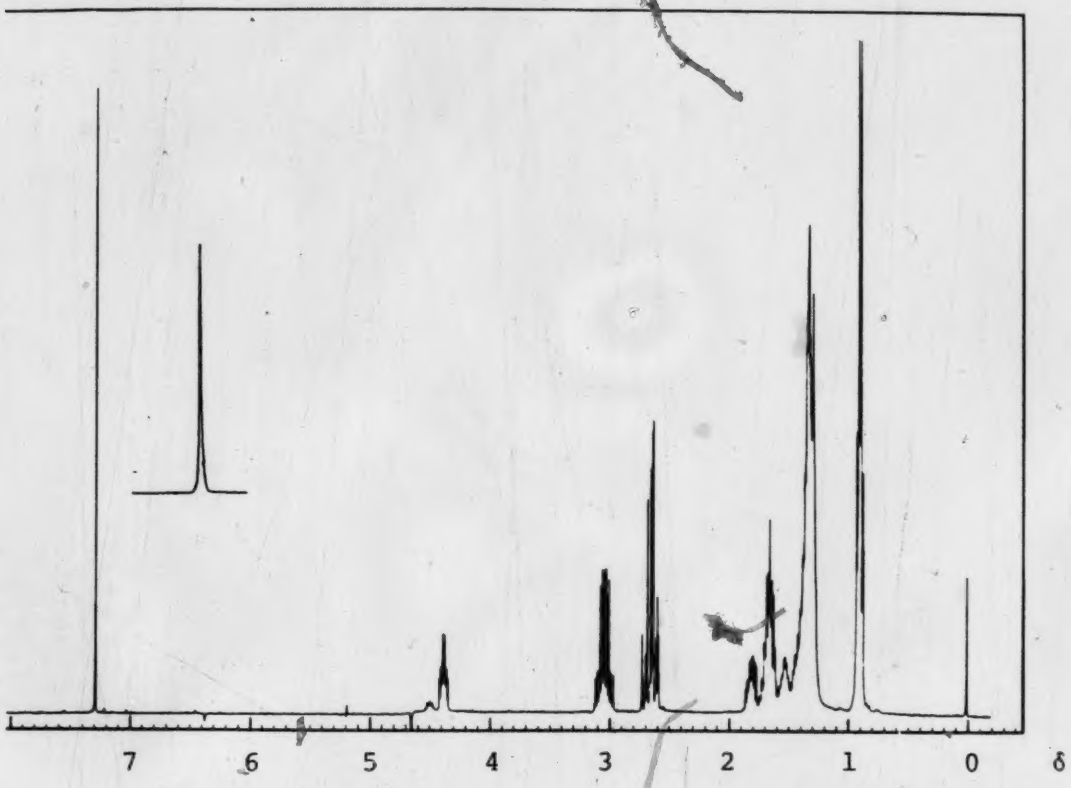


FIGURE 2: 400 MHz ^1H NMR spectrum ($CDCl_3$) of Lachnelluloic Acid (6).

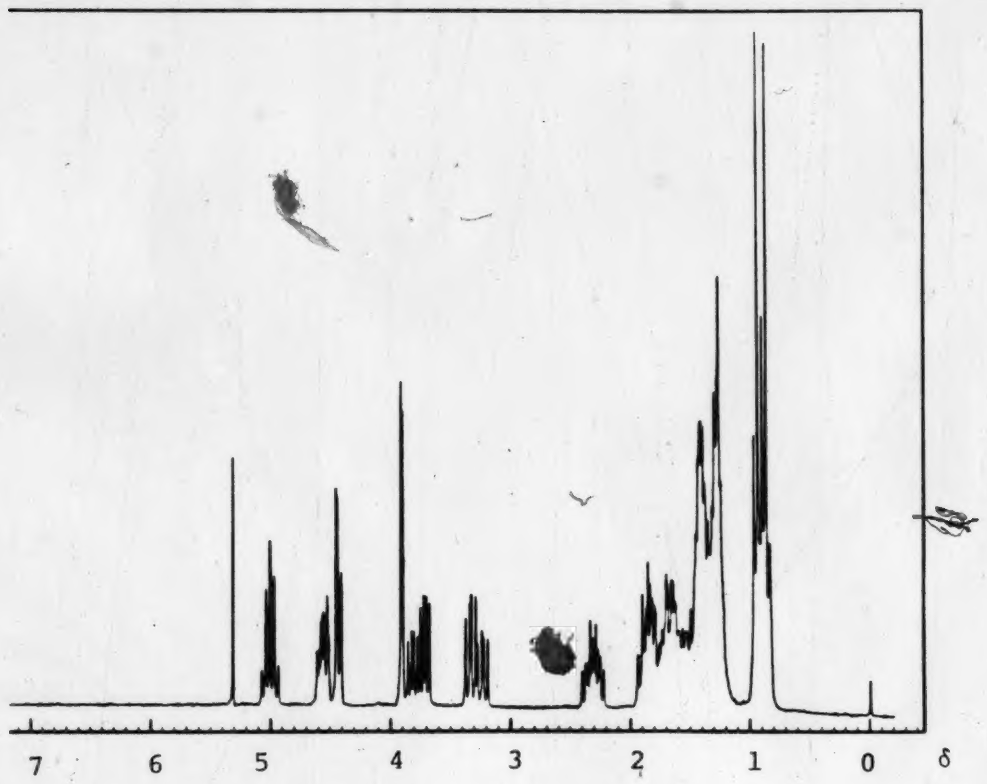


FIGURE 3: 200 MHz ^{1}H NMR spectrum ($CDCl_3$) of Lachnellulone (III or V).

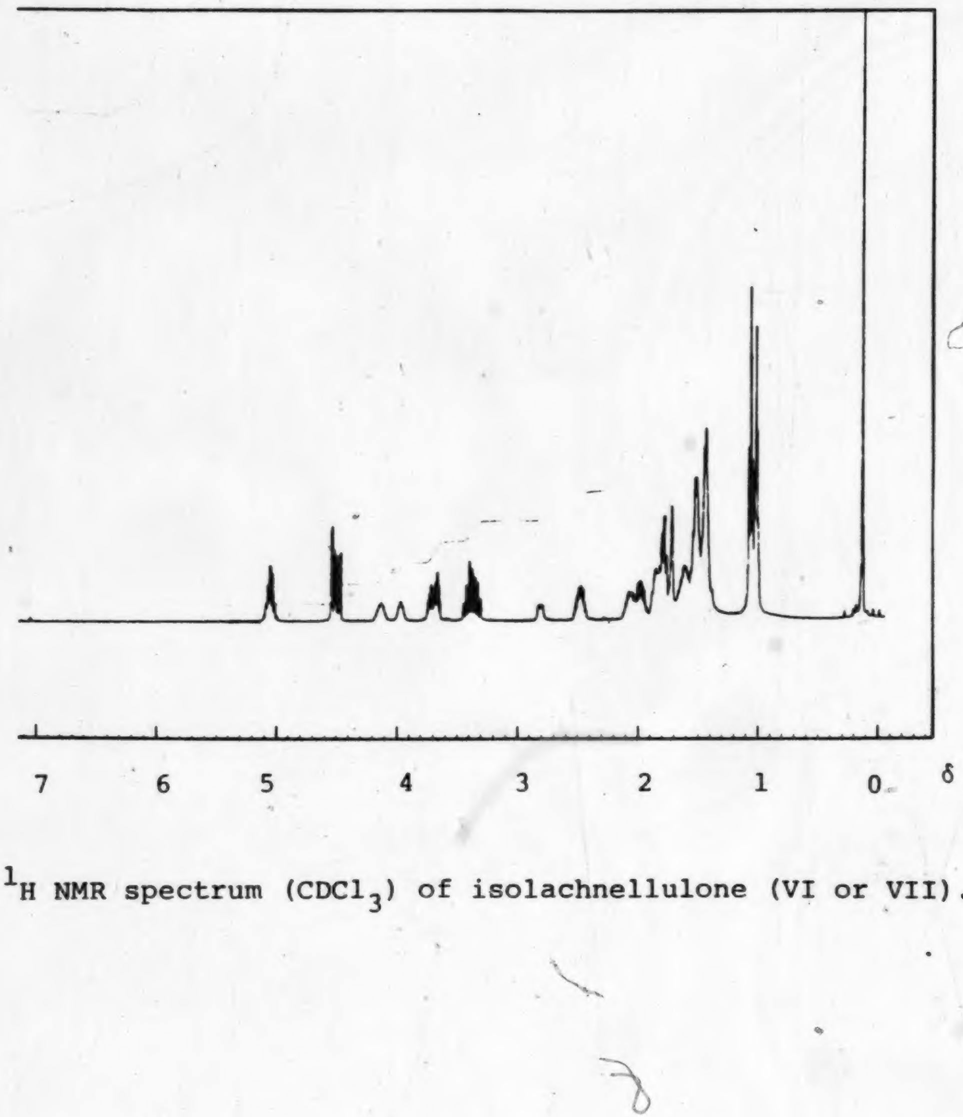


FIGURE 4: 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of isolachnellulone (VI or VII).

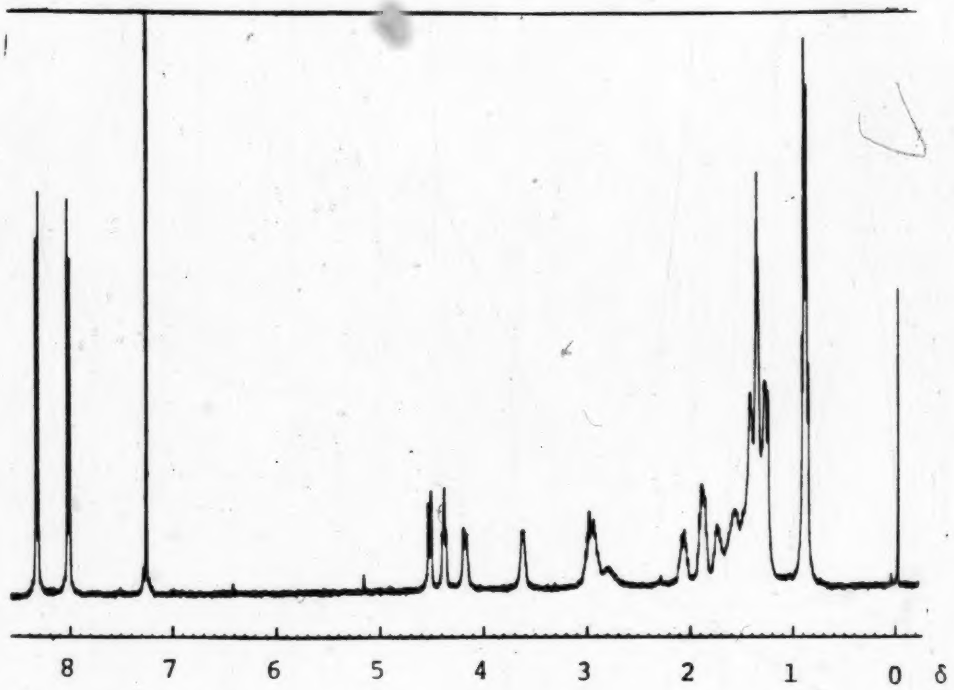


FIGURE 5: 400 MHz ^1H NMR spectrum ($CDCl_3$) of Lachnellulone-p-nitrophenyl hydrazone.

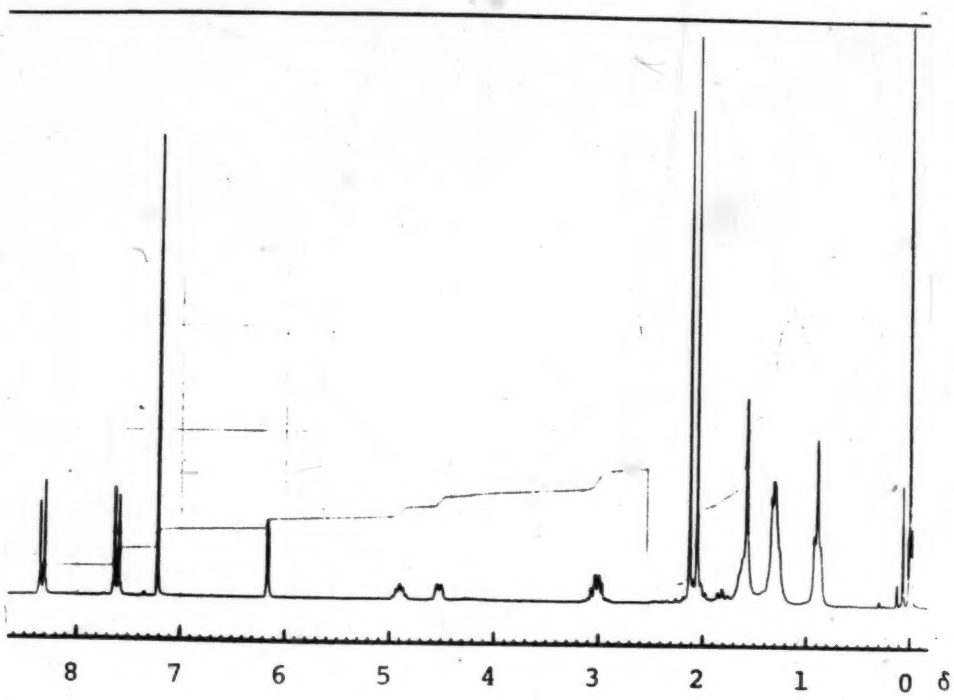


FIGURE 6: 200 MHz ^{1}H NMR spectrum ($CDCl_3$) of Lachnellulone-p-nitrophenyl hydrazone diacetate.

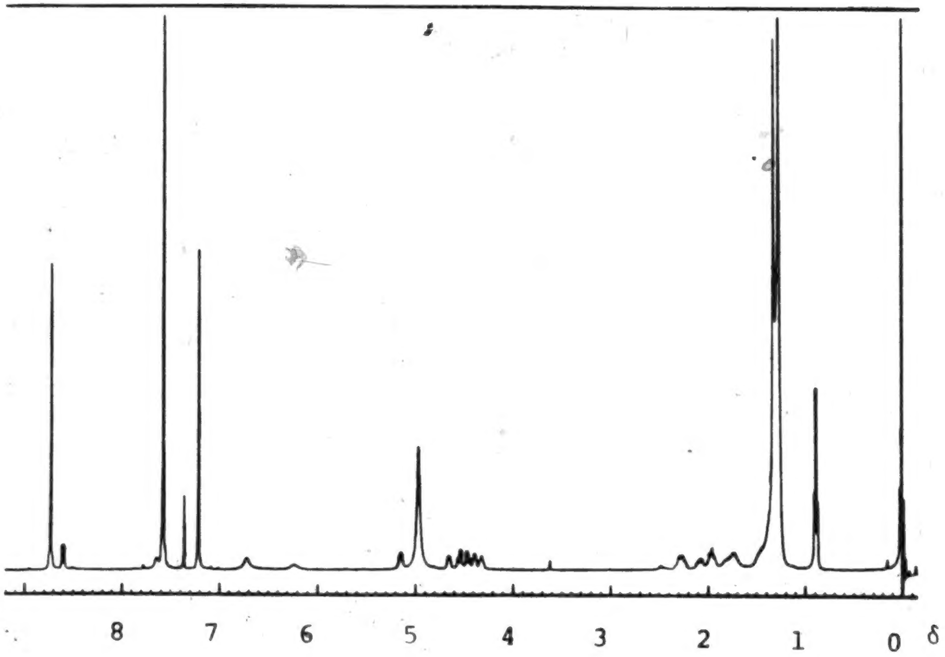


FIGURE 7: 400 MHz ^{1}H NMR spectrum (Py-$\underline{d}_5$) of the nitrogen containing metabolite.

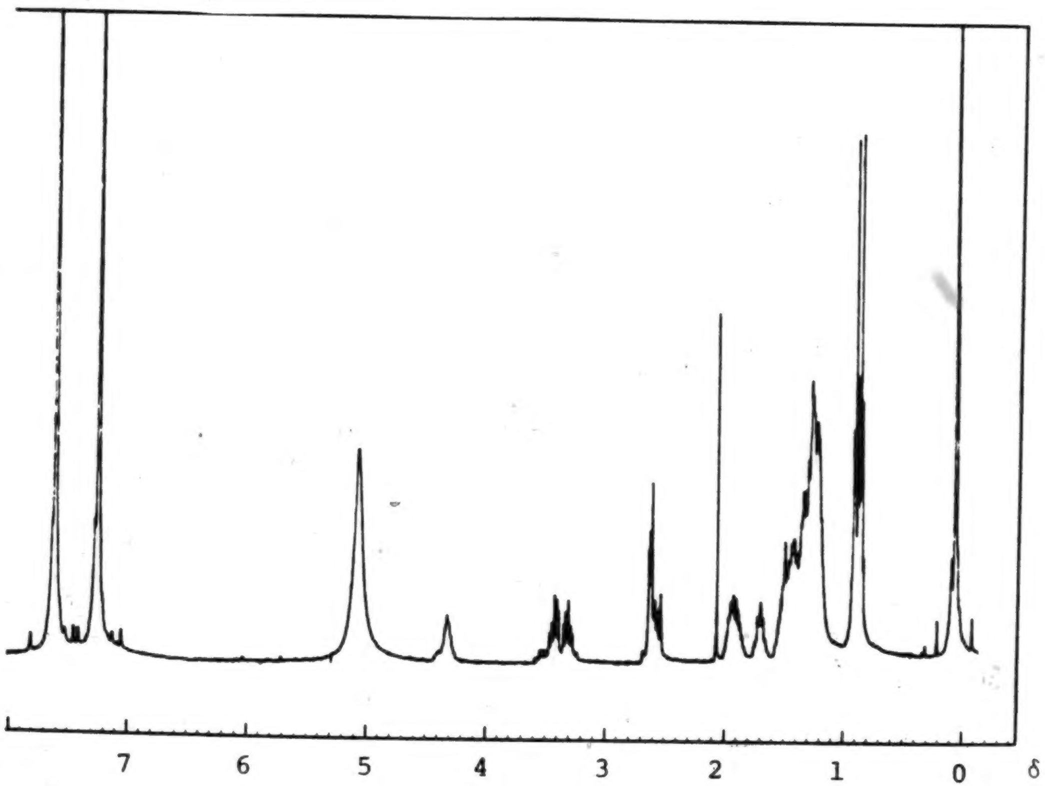


FIGURE 8: 400 MHz ^{1}H NMR spectrum (pyridine-$\underline{d}_5$) of compound T-1.

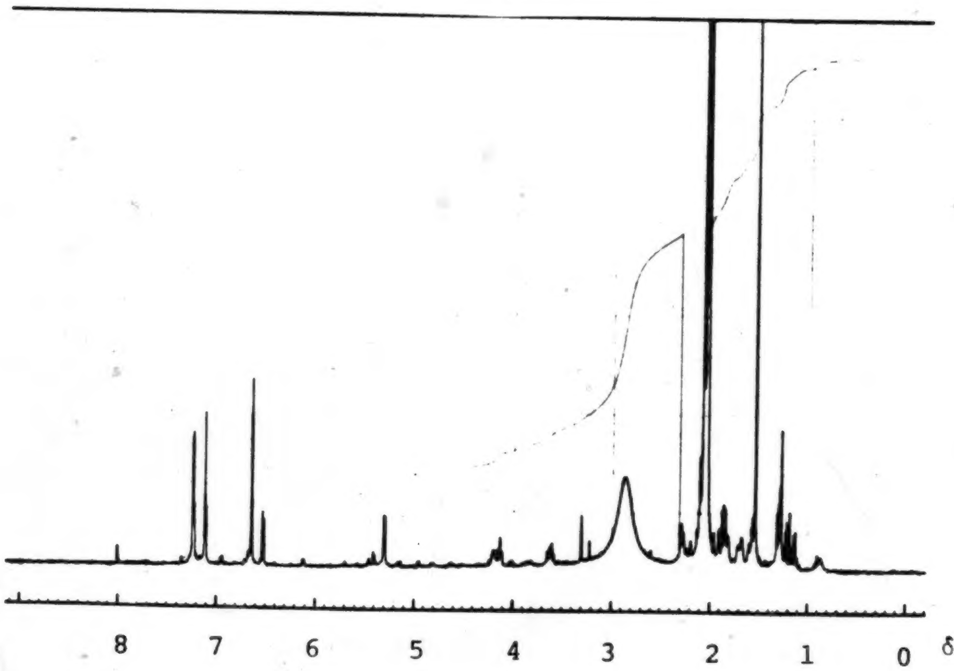
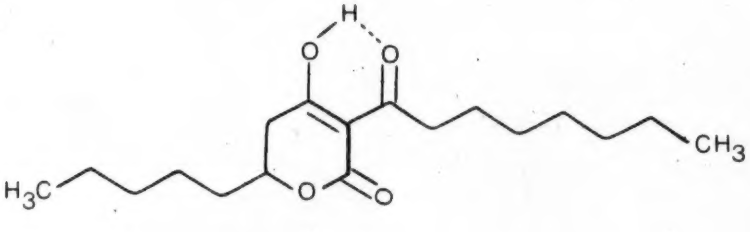


FIGURE 9: 400 MHz ^1H NMR spectrum (acetone-$\underline{d}_6$) of the yellow dye.

CHAPTER 2

The Total Synthesis of Lachnelluloic Acid

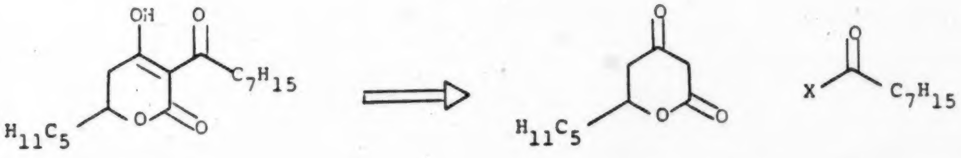
As was discussed in Chapter I, lachnelluloic acid is believed to possess structure **1**.


H
O
O
CH3
H3C
O
O

1

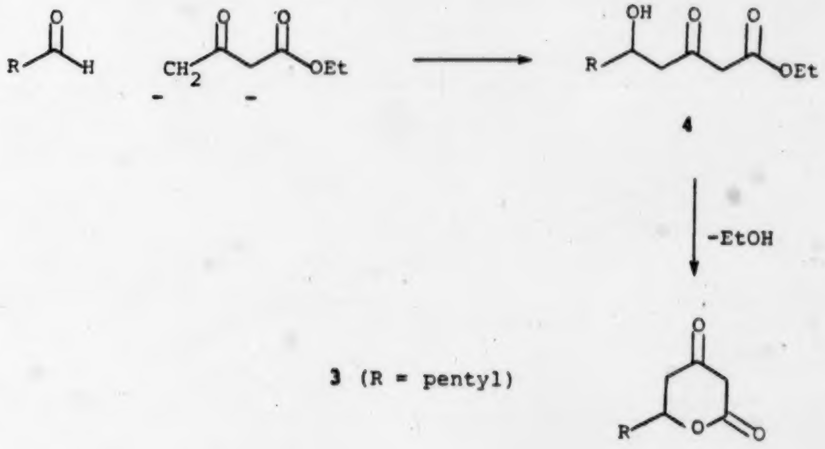
In order to develop a synthetic approach to this molecule several retrosynthetic analyses were considered. Scheme 1 shows the most obvious approach, which involves the acylation of a β-ketolactone.

SCHEME 1:

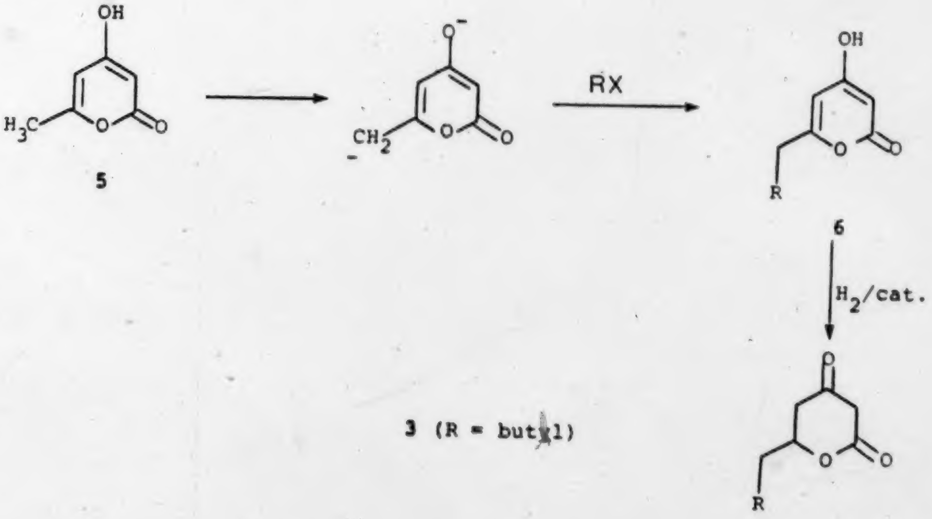

OH
O
C7H15
H11C5
O
O
O
H11C5
O
O
O
X
C7H15

1 **2** **3**

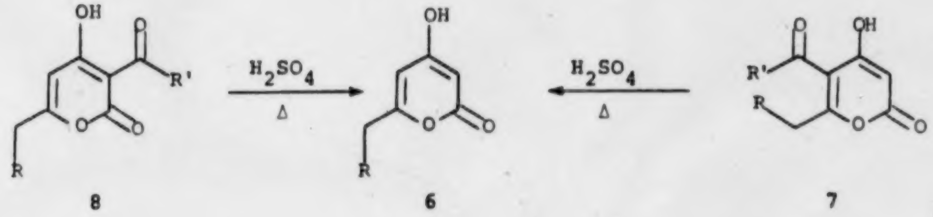
β-Ketolactone **2** and its analogues may be obtained in several different ways: 1 - by condensation of the dianion of ethyl acetoacetate with suitable aldehydes[1] followed by lactonization;


R
H
CH2
OEt
OH
4
-EtOH
3 (R = pentyl)
O

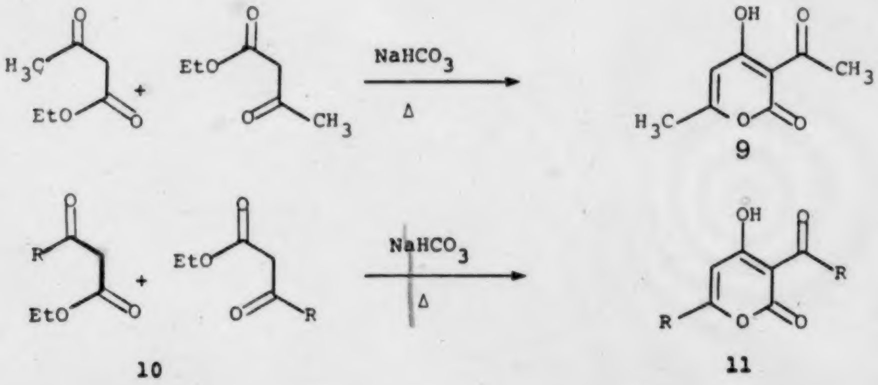
2- by alkylation of the triacetic acid lactone **5**[2] followed by hydrogenation of the intermediate 4-hydroxy-6-alkyl-2-pyrone (**6**).[3]


OH
H3C
5
CH2
RX
R
6
H2/cat.
3 (R = butyl)

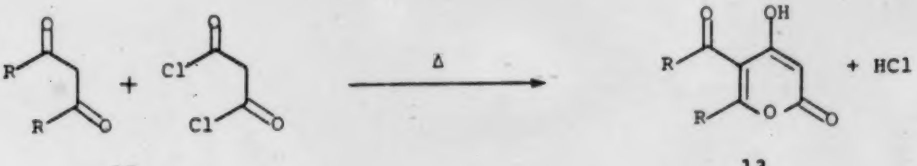
The intermediate **6** may also be obtained by acid-catalyzed deacylation of the 3- or 5-acyl-4-hydroxy-2-pyrones, **7** or **8**, respectively.[4]


OH
R'
R
H_2SO_4
Δ
8
6
7

The 3-acyl pyrones (**8**) may be synthesized in a manner similar to that used for the preparation of dehydroacetic acid (**9**),[5] by condensation of two appropriately substituted β-ketoesters.

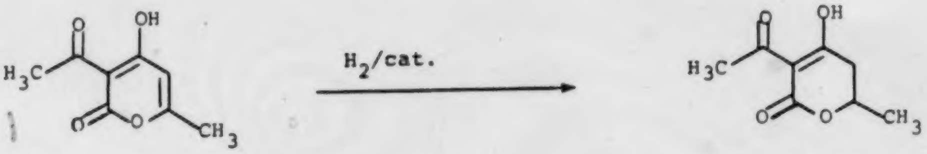

H_3C
EtO
CH_3
$NaHCO_3$
Δ
OH
9
R
10
11

The 5-acyl-pyrones **7** may be prepared by Elvidge's procedure,[6] that is, by condensation of malonyl dichloride with a β-diketone.

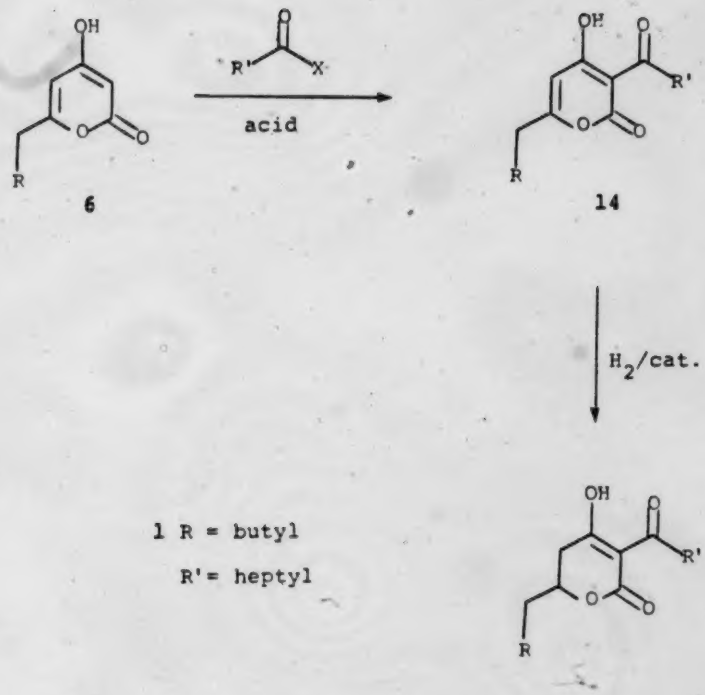


12 **13**

Another interesting approach to the synthesis of lacnelluloic acid (**1**) involves the conversion of an intermediate pyrone **6** to a dehydroacetic acid analogue **14** followed by hydrogenation in a manner analogous to that used in the preparation of dihydrodehydroacetic acid (**15**).



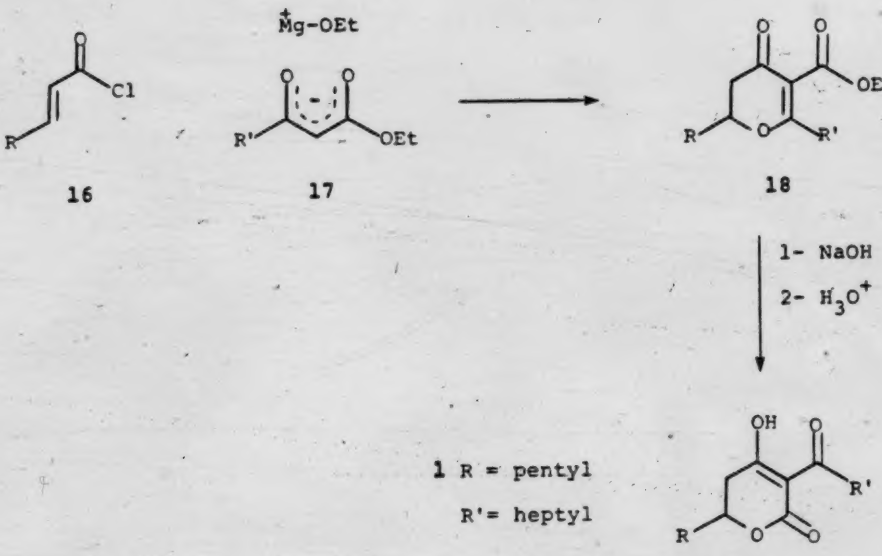
9 **15**



1 R = butyl
R'= heptyl

The advantage of this approach is the facility with which 6-alkyl-4-hydroxy-2-pyrones such as **6** undergo direct C-acylation under acid catalysis.[7] The direct C-acylation of β-ketolactones proceeds in poor yield under acid catalysis.[8]

A third approach involves the base-catalyzed rearrangement of 3-carbethoxy-2,6-dialkyl-5,6-dihydro-4-pyrones (**18**).[9] Compound **18** may be obtained by treatment of an α,β-unsaturated acid chloride **16** with the ethoxy magnesium enolate of a β-ketoester **17.**

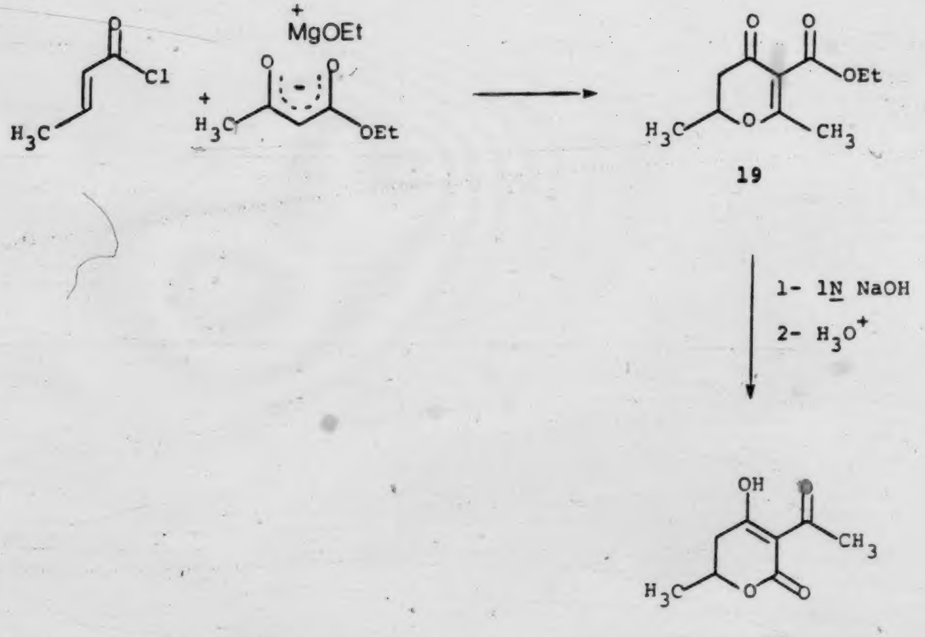


16 17 18

1 R = pentyl
R'= heptyl

Lachnelluloic acid (**1**) and structural analogues should be readily available by using one of these synthetic approaches through the variation of groups R and R'.

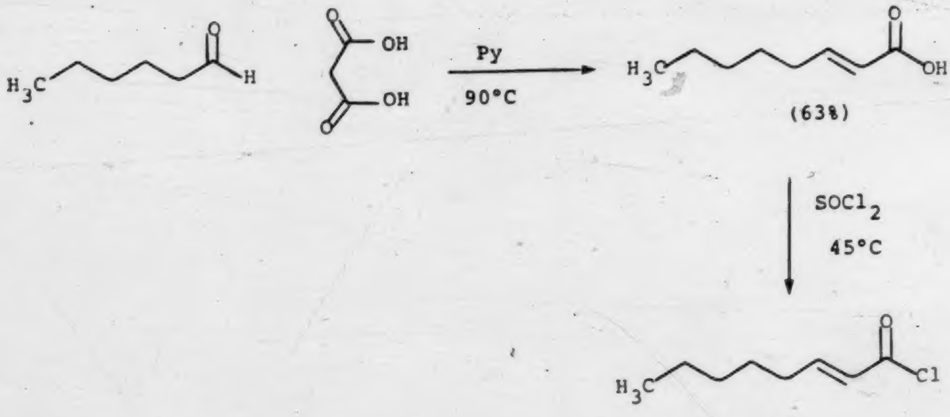
In 1968, Gelin[9] reported an elegant synthesis of dihydrodehydroacetic acid (**15**). His approach consisted of the treatment of the ethoxy magnesium enolate of ethyl acetoacetate with crotonyl chloride to produce 3-carboethoxy-2,6-dimethyl-5,6-dihydro-4-pyrone (**19**). Compound **19** is easily rearranged to dihydrodehydroacetic acid (**15**) by treatment with dilute aqueous sodium hydroxide at room temperature, followed by acid work up.



19

15

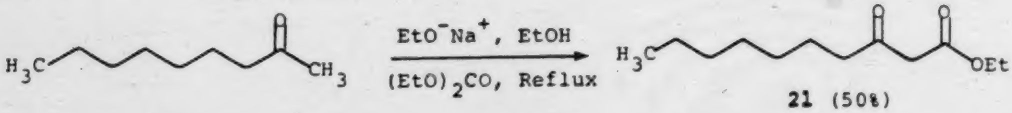
After successfully repeating this synthetic process it was felt that lachnelluloic acid could be prepared following Gelin's sequence, provided the side chain size in the α,β-unsaturated acid chloride and in the β-ketoester could be controlled. The required acid chloride, trans-2-octenoyl chloride (**20**), was prepared from the commercially available acid or from trans-2-octenoic acid obtained by Doebner condensation of hexanal with malonic acid in pyridine.[10] Treatment of trans-2-octenoic acid with thionyl chloride at 50°C for 45 minutes produced a dark brown solution which afforded trans-2-octenoyl chloride (**20**, IR 1750 cm^{-1}) as a colorless liquid after distillation under reduced pressure.



H_3C
H
OH
OH
Py
90°C
H_3C
OH
(63%)
$SOCl_2$
45°C
H_3C
Cl

20 (65%)

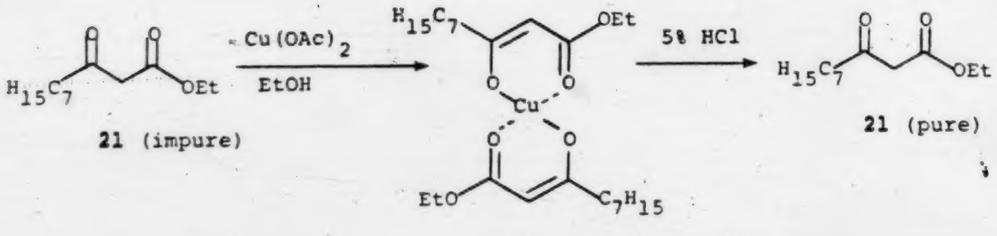
The desired β-ketoester, ethyl 3-oxodecanoate (**21**), was prepared by condensation of 2-nonanone with diethyl carbonate in the presence of sodium ethoxide.[11]



H_3C
CH_3
EtO^-Na^+, EtOH
$(EtO)_2CO$, Reflux
H_3C
OEt

21 (50%)

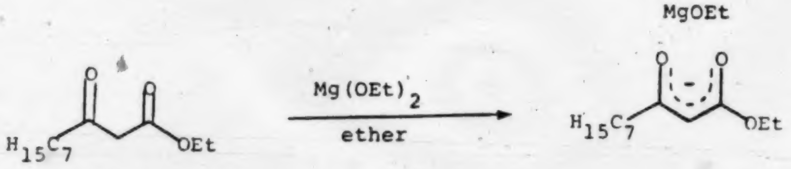
This β-ketoester was purified by complex formation with copper acetate in 95% ethanol.[12] The copper complex **22** was washed with cold 95% ethanol and finally decomposed with acid to afford the pure β-ketoester **21** as a colorless liquid with a characteristic odor. It shows absorptions in the IR spectrum at 1740 cm^{-1} (-COOR) and 1715 cm^{-1} (ketone).



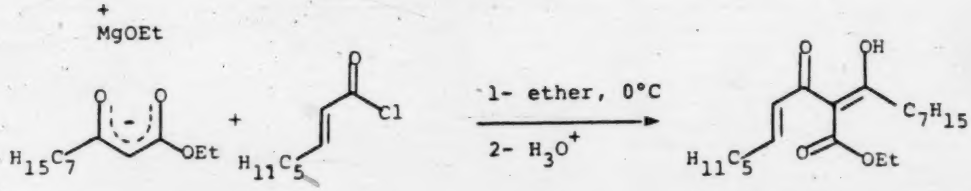
22

The ethyoxymagnesium salt of the β-ketoester **21** was prepared by mixing **21** with an ethereal solution of magnesium ethoxide (obtained from dry ethanol and magnesium metal). Slow addition of trans-2-octenoyl chloride to the solution of the ethoxymagnesium enolate at 0°C afforded, after aqueous sulfuric acid work up, a yellow oil which on vacuum distillation yielded a single product. This material was identified as the open form of the desired 3-carboethoxy-2-heptyl-6-pentyl-5,6-dihydro-2-pyrone, compound **23**, by its ^1H NMR spectrum (δ 17.4 (s, 1H), 7.0 (m, 1H), 6.4 (bd, 1H)), and by virtue of the fact that it could be converted to the closed form **24** under a variety of conditions.

$$\mathrm{Mg^\circ + EtOH \xrightarrow[\Delta]{CCl_4\ (cat.)} Mg(OEt)_2 + H_2}$$


H15C7
OEt
Mg(OEt)2
ether
+-
MgOEt
H15C7
OEt

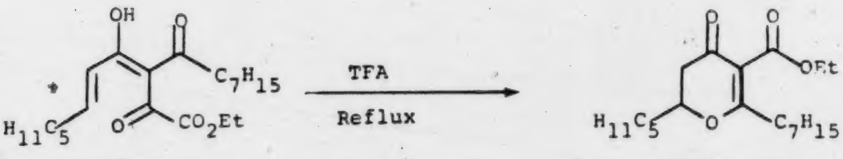
21


+
MgOEt
H15C7
OEt
+
Cl
H11C5
1- ether, 0°C
2- H3O+
OH
C7H15
H11C5
OEt

20

23 (~100%)

The tricarbonyl compound **23** could be converted to the desired dihydropyrone **24** by treatment with trifluoroacetic acid under reflux.


OH
C7H15
H11C5
CO2Et
TFA
Reflux
OEt
H11C5
C7H15

23

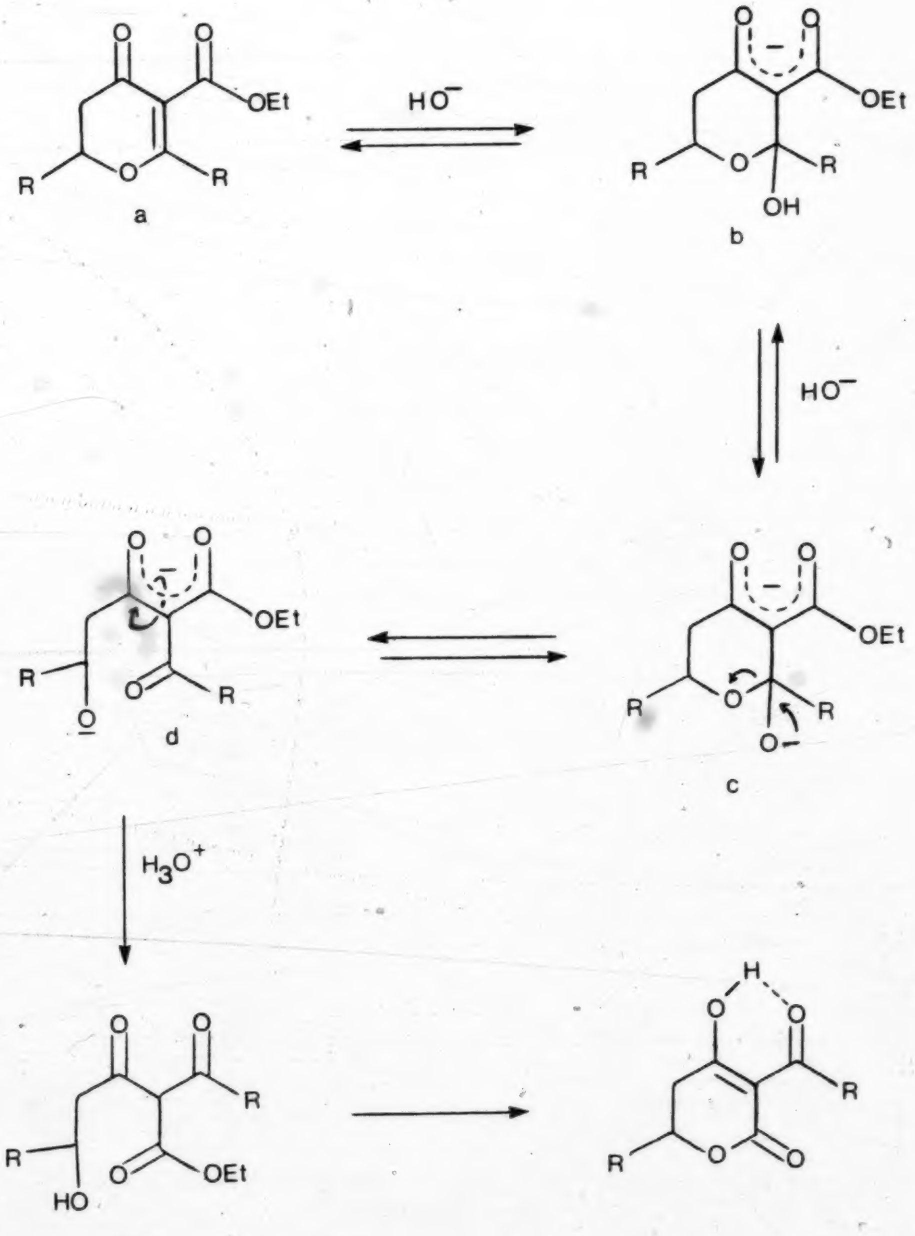
24 (69%)

The dihydropyrone **24** was purified by flash chromatography to give a colorless liquid (MW 328 ($C_{20}H_{34}O_4$, 100%), IR 1710, 1670 and 1585 cm^{-1}, ^1H NMR δ 4.40 (1H, m), UV λ_{max} 266 nm). Its structure was confirmed by comparison of its spectral characteristics with those of the intermediate in Gelin's synthesis, compound **19**.

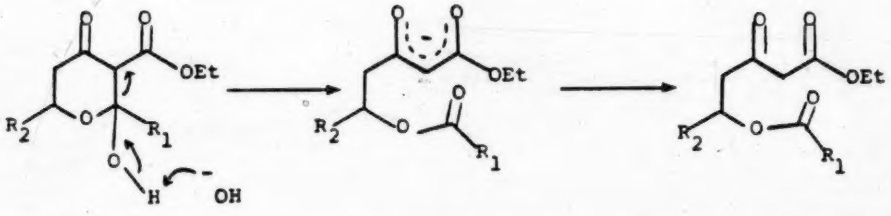
Unfortunately, when the dihydropyrone **24** was treated with 1 M NaOH for 1,3 or 10 h at room temperature followed by acidification, no trace of lachnelluloic acid was detected. An attempted rearrangement with ethanolic NaOH gave the same negative result. Only the starting material, **24**, and its open form **23**, along with small amounts of decomposition products, were detected in the reaction mixture.

The mechanism proposed for the rearrangement reaction is shown in Scheme 2.

Scheme 2:


O
O
OEt
R
O
R
a
HO⁻
O
O
OEt
R
O
R
OH
b
HO⁻
O
O
OEt
R
O
O
R
d
O
O
OEt
R
O
R
O⁻
c
H3O+
O
O
R
R
HO
O
OEt
e
H
O
O
R
R
O
O

Two equivalents of base are required for the rearrangement in order to obtain the dianionic intermediate **c**. With one equivalent of base intermediate **b**, or its protonated form **b'** are formed, which can then fragment leading to the formation of the diester **f**.

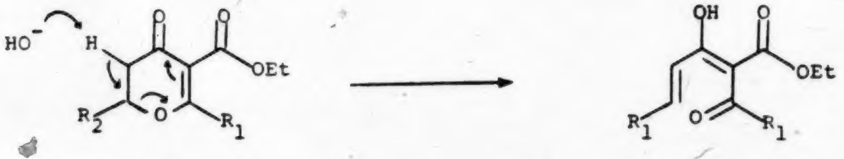


b'

f

With this proposed mechanism in mind, there are two possible explanations for the failure of the dihydropyrone **24** to rearrange.

1. A base-catalyzed ring opening of the dihydropyrone **24** to give **23** takes place instead of 1,4-addition of the hydroxyl ion.



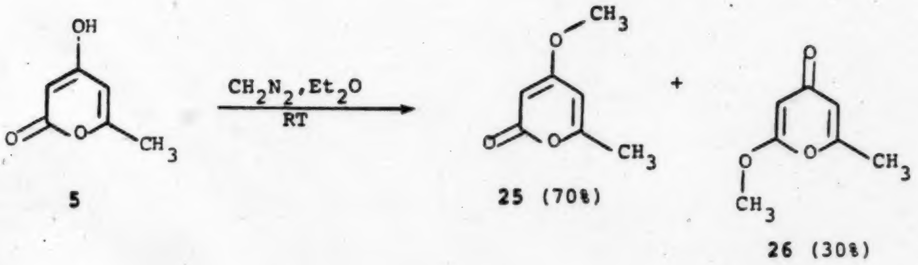
24

23

2. The size of the side chains R_1 and R_2 may hinder the desired C-C rotation indicated in the proposed intermediate **d** (Scheme 2).

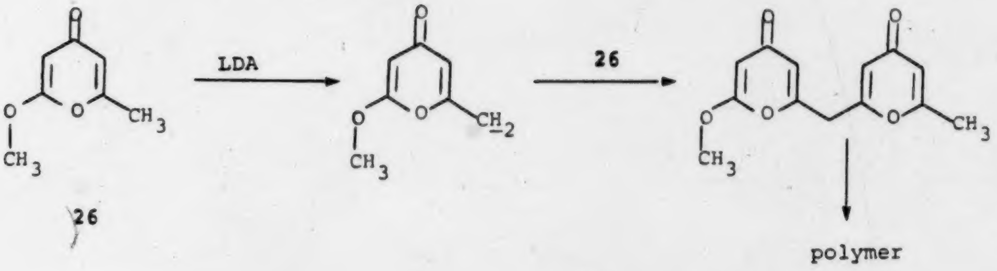
Another interesting approach to the synthesis of lachnelluloic acid is that which involves a dehydroacetic acid analogue such as **14.** This approach consists of the acylation of an intermediate such as **6,** a process that proceeds smoothly and in high yield[7] (see page 126). Since lachnelluloic acid analogues can be oxidized to dehydroacetic acid analogues, and dehydroacetic acid is known to possess bactericidal, fungicidal, and renal tube blocking activity,[13] it is reasonable to propose that dehydrolachnelluloic acid **14** (R = pentyl, R' - heptyl) is a physiologically active form of lachnelluloic acid.

Initial attempts to obtain the key intermediate, lactone **6** (R = n-butyl), involved the alkylation of a triacetic acid lactone derivative. It is known that triacetic acid lactone (**5**) undergoes reaction with ethereal diazomethane to produce two main compounds: the 2-methoxy- and 4-methoxy- derivatives **25** and **26**, respectively.[14] In our hands, these two products were obtained in a 7:3 ratio, with the pyrone **25** as the major component.



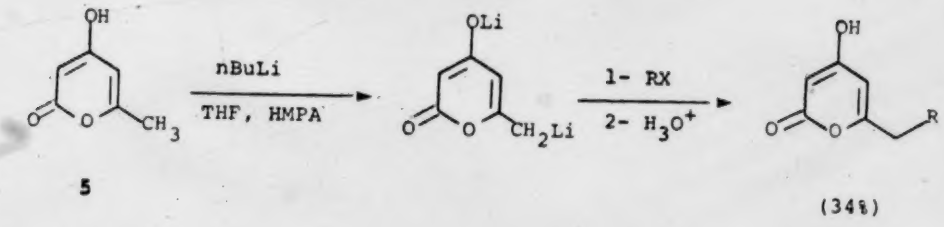
The reaction mixture was separated by flash chromatography and each compound was identified by its spectroscopic properties, especially by their UV spectra. The least polar and more abundant product was identified as **25** (λ_{max} 280 nm doubly conjugated lactone),[15] while structure **26** was assigned to the most polar compound (λ_{max} 245 nm, 4-pyrone).

2-Methoxy-6-methyl-4-pyrone (**26**) was considered to be the most convenient substrate for the alkylation reaction because there are more precedents for δ-alkylations than for ε-alkylations.[16] When the pyrone **26** was treated with one equivalent of either lithium diisopropylamide (LDA) or nbutyllithium (n-BuLi) in THF at -78°C, a deep red solution was obtained. This was initially thought to contain the enolate, however, the color remained unchanged even after treatment with excess methyl iodide or heavy water. TLC analysis of the colored solution indicated the presence of a complex mixture of compounds. It is possible that pyrone **26** is very active toward 1,4-addition of nucleophiles, and readily forms polymerization products.

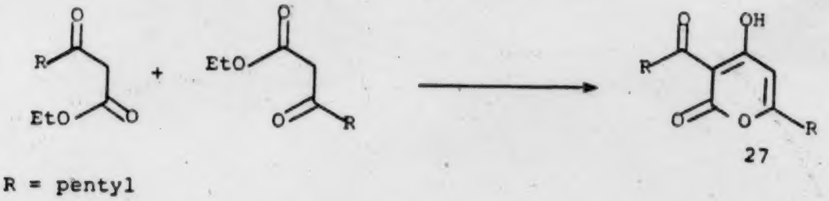


26

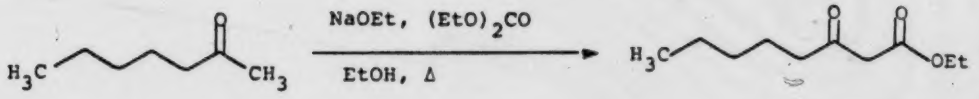
After several unsuccessful experiments this method of preparation of **6** was abandoned. [After the synthesis of lachnelluloic acid was completed we discovered literature precedents for the alkylation of triacetic acid lactone (**5**) by means of its dilithium salt[2]].



Another method to prepare the lactone **6** involves the condensation of two molecules of a suitably substituted β-ketoester to produce 3-acyl-4-hydroxy-6-pentyl-2-pyrones **27**, which could be deacylated to pyrone **6**.

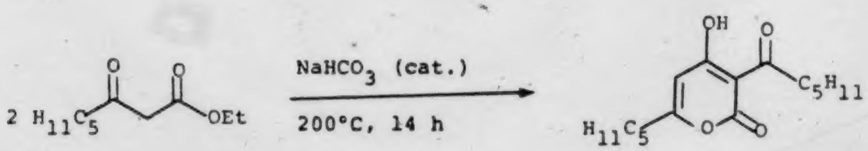


The desired β-ketoester **28** was prepared by reaction of the sodium enolate of 2-heptanone with diethyl carbonate. The ethanol produced during the reaction was continuously removed by distillation.[11] Ethyl 3-oxooctanoate (**28**) was purified by vacuum distillation followed by complexation-decomplexation with copper acetate. Pure β-ketoester **28** is a colorless liquid (IR 1740, 1715 cm^{-1}, MW 188 ($C_{10}H_{18}O_3$)).


H3C
CH3
NaOEt, (EtO)2CO
EtOH, Δ
H3C
OEt

28 (50%)

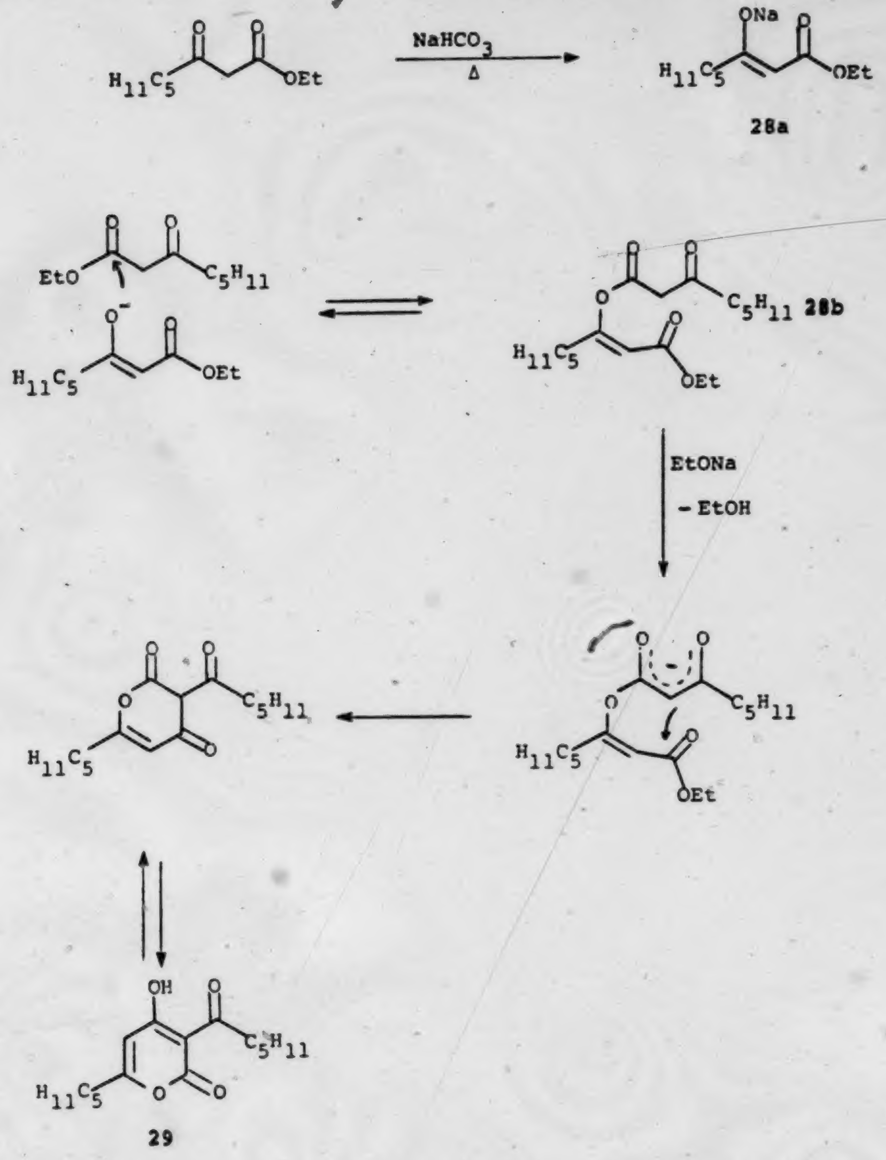
The autocondensation reaction was carried out by heating neat β-ketoester **28** at 200°C for 14 h in the presence of a catalytic amount of sodium bicarbonate. The dark reaction mixture obtained was partially purified by flash chromatography, and the fractions containing the desired product were recrystallized from Skellysolve B to afford white crystals of 3-hexanoyl-4-hydroxy-6-pentyl-2-pyrone (**29**) (MW 280 ($C_{16}H_{24}O_4$), IR 1720, 1635 cm^{-1}, ^{1}H NMR δ 12.1 (1H), 6.0 (1H)).


2 H11C5
OEt
NaHCO3 (cat.)
200°C, 14 h
OH
C5H11
H11C5

28

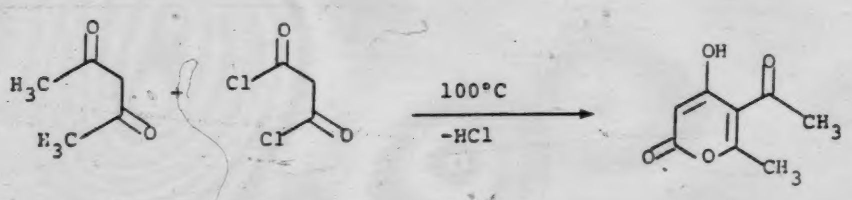
29 (20%)

The condensation is probably initiated by reaction of sodium bicarbonate (or the sodium carbonate derived from it) with the β-ketoester to produce a sodium enolate **28a** as shown below. Enolate **28a** undergoes reaction with another molecule of β-ketoester to produce enol ester **28b** by elimination of sodium ethoxide. The sodium ethoxide liberated catalyzes ring formation by a Dieckmann

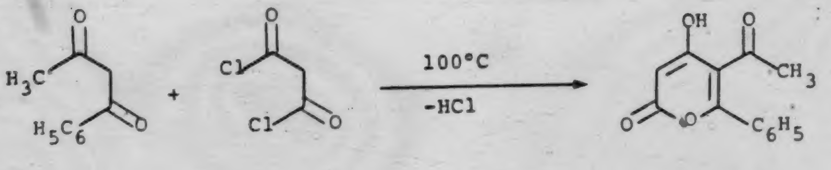


condensation. The low yield of this condensation reaction (20%) led us to investigate other synthetic approaches to lactone **6**.

The preparation of lactone **6** was next attempted using the condensation of β-diketones with malonyl dichloride. Elvidge and co-workers report that the reaction of β-diketones with malonyl dichloride produces 5-acyl-6-alkyl-4-hydroxy-2-pyrones.[6] They have prepared 5-acetyl-4-hydroxy-6-methyl-2-pyrone (**30**) and 5-acetyl-4-hydroxy-6-phenyl-2-pyrone (**31**) from acetylacetone and benzoylacetone, respectively.

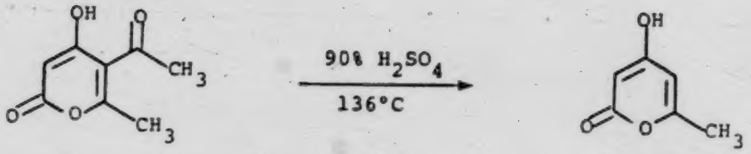


30 (42%)



31 (50%)

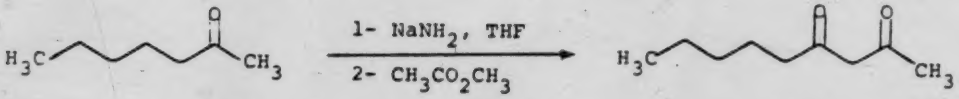
The structure of pyrone **30** was proven by deacylation to triacetic acid lactone **5** and acetic acid.



30 **5** (75%)

The use of this method to prepare the desired lactone **6** (R = butyl) requires a β-diketone containing a n-pentyl side chain.

The unsymmetrical β-diketone 2,4-nonadione (**32**) was synthesized by sodium amide catalyzed condensation of 2-heptanone with methyl acetate in THF.[17]

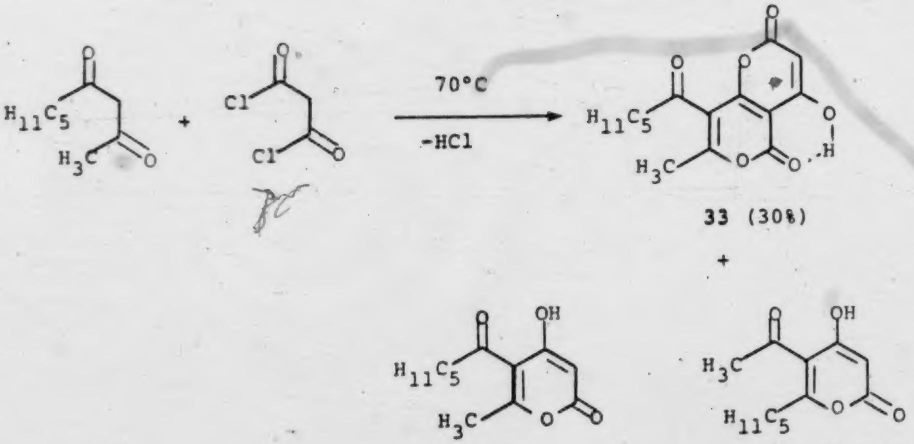


32 (51%)

This β-diketone was purified by a complexation-decomplexation sequence with copper acetate to give **32** as a colorless oil (n_D^{20} 1.458, IR 1728, 1708, 1615 cm^{-1}).

Equimolecular amounts of the β-diketone **32** and freshly prepared malonyl dichloride[18] were mixed, and the resulting solution was heated to 70°C for 10 minutes. The hydrochloric acid produced was removed continuously by means of a water aspirator. Flash chromatography of the dark brown reaction mixture afforded two components (by TLC). The least polar component was a white crystalline material (mp 119-121°C) which contained three more carbons and two more oxygen atoms than the expected product. The ^1H NMR spectrum for this compound shows the presence of an enolic hydroxyl (δ 10.4), a single vinylic proton (δ 5.6) and an aromatic methyl group (δ 2.4 (3H)). We believe that this compound is produced by reaction of one molecule of the β-ketoester **32** with two molecules of malonyl dichloride and that it possesses structure **33**.

The most polar component was isolated as an amorphous solid (MW 224 ($C_{12}H_{16}O_4$)). Its ^1H NMR spectrum is similar to that of compound **33** (δ 11.2 (1H), 5.5 (1H), 2.9 (2H, t), 2.7 (3H, s)). Since there are two possible modes of reaction between the β-diketone **32** and malonyl dichloride, it was concluded that this component was a mixture of compounds **34** and **35**.



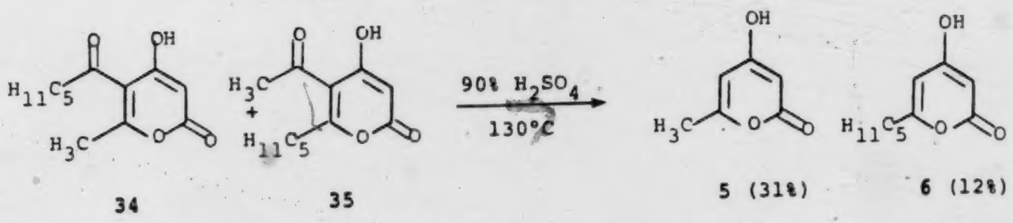
33 (30%)

34

35

(30% **34** + **35**)

As expected, when the mixture of compounds **34** and **35** was treated with 90% H_2SO_4 at 130°C two products were obtained, which were separated by flash chromatography. The least polar component, present in minor amount, was shown to be the desired lactone **6** (R = butyl) (MW 182 ($C_{10}H_{14}O_3$), ^1H NMR δ 10.9 (1H, s), 6.1 (1H, s), 5.6 (1H, s)). The major product of the deacylation reaction was a crystalline solid (mp 109-112°C, MW 168 ($C_8H_6O_3$)) which was identified as triacetic acid lactone (**5**).

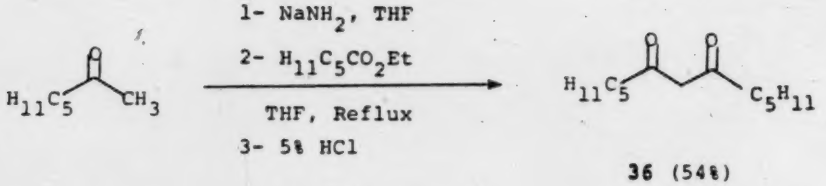


34 **35**

The desired lactone **6** is produced by deacylation of compound **35** while triacetic acid lactone (**5**) is formed when the pyrone **34** is deacylated. Thus, the desired product, 5-acetyl-4-hydroxy-6-pentyl-2-pyrone (**35**), is the minor product of the condensation reaction.

To avoid this problem we used a symmetrical β-diketone in the condensation with malonyl dichloride.

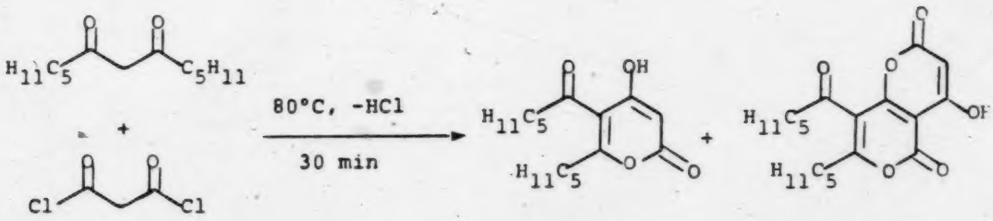
The desired β-diketone; 6,8-tridecadione (**36**), was prepared from 2-heptanone and methyl hexanoate in the usual way, that is, by treatment of the sodium enolate of 2-heptanone with methyl hexanoate in refluxing THF.[17] After work up, the reaction mixture was distilled under reduced pressure to afford impure β-diketone, which was further purified by the complexation - decomplexation process with copper acetate to give **36** as a colorless liquid (n_D^{20} 1.462, MW 212 ($C_{13}H_{24}O_2$), IR 1705, 1610 cm^{-1}).



36 (54%)

When equimolecular amounts of the diketone **36** and malonyl dichloride were mixed and heated to 80°C, a copious amount of hydrogen chloride was produced. The reaction was worked up once the evolution of HCl had stopped. Recrystallization of the reaction mixture afforded a pale yellow crystalline material (mp 91-92°C) which was identified as the desired product **37** on the basis of its spectral characteristics; MW 180 ($C_{16}H_{24}O_4$), ^1H NMR δ 16.71 (1H, s), 5.56 (1H, s), 2.82 (2H, t), 2.73 (2H, bt), 1.70 (4H, m), 1.31 (8H, m) and 0.90 (6H, vt).

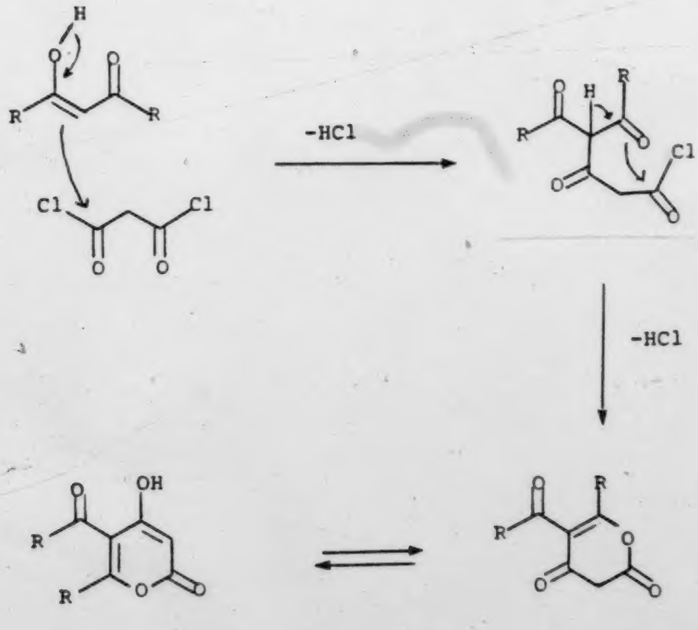
A second product of this reaction was obtained after purification of the mother liquors by flash chromatography. This pale yellow crystalline material (mp 80-82°C) is believed to be the product of reaction of **37** with another molecule of malonyl dichloride. Its HREIMS; MW 348 ($C_{19}H_{24}O_6$) and its ^1H NMR spectrum, δ 10.4 (1H, s), 5.6 (1H, s), 2.9 (2H, t), 2.6 (2H, t), 1.7 (4H, m), 1.4 (8H, m) and 0.9 (6H, vt) are consistent with structure **38**.


80°C, -HCl
30 min

37 (60%) **38** (10%)

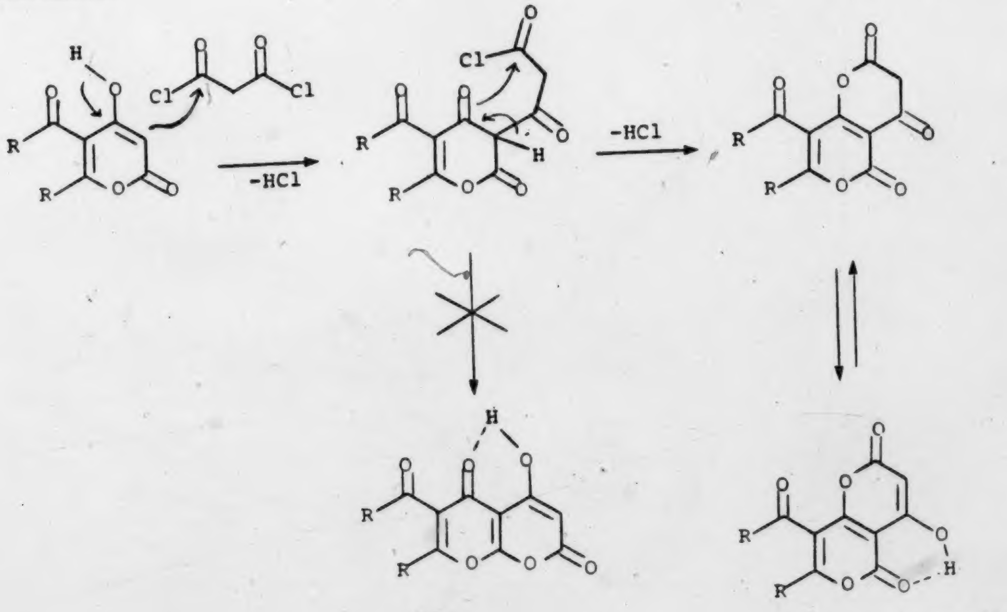
This reaction probably proceeds by C-acylation of the β-diketone followed by enol ether formation, as indicated in Scheme 3.

SCHEME 3:



The bicyclic product **38** is formed by further C-acylation with malonyl dichloride, as shown below.

SCHEME 4:

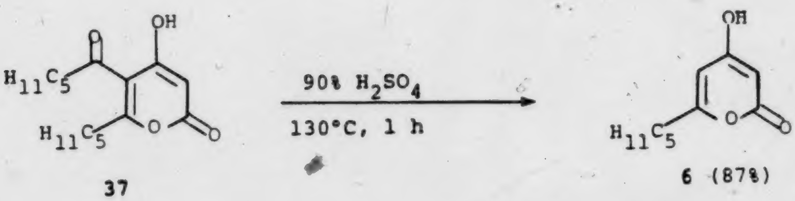


38a

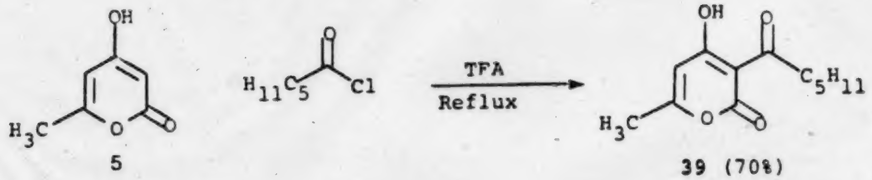
38 (R = pentyl)

Subsequent ring closure occurs in such a way that compound **38** rather than **38a** is formed. This is because enolization in the monocyclic intermediate takes place preferentially at the ketone carbonyl and not at the lactone carbonyl. This assumption is supported by the absence of a very low field hydrogen bonded hydroxyl proton (e.g., δ 16 - δ 18) in the ^{1}H NMR, and the absence in the ^{13}C NMR spectrum of the compound of signals at 90-100 ppm and 175-185 ppm, which would be expected for the highly polarized ring junction carbons in compound **38a**.

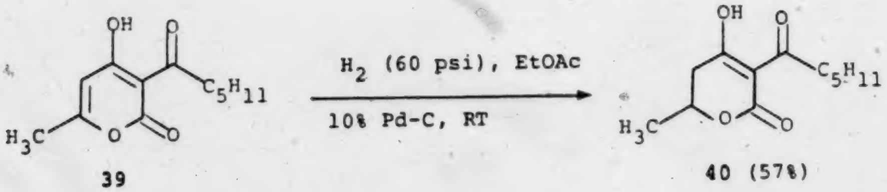
Pyrone **37** was deacylated by treatment with 90% H_2SO_4 at 130°C. Recrystallization or flash chromatography of the crude reaction mixture gave a white crystalline compound (mp 52-52.5°C) which was identified as the lactone **6** (see page 144). This deacylation occurred in 87% yield. The overall yield of **6** from the β-diketone **36** is 52% (compare with the overall yield of Poulton's procedure, 34%).[2]



To test the reliability of the acylation reaction, triacetic acid lactone (**5**) was treated with hexanoyl chloride in refluxing trifluoroacetic acid (TFA).[7] Flash chromatography of the reaction mixture afforded white crystals (mp 59-60°C) of a material that was identified as 3-hexanoyltriacetic acid lactone (**39**) by comparison with its analogue, dehydroacetic acid (**9**); MW 224 ($C_{12}H_{16}O_4$); ^1H NMR δ 16.90 (1H, s), 5.98 (1H, s), 3.10 (2H, t), 2.30 (3H, s), 1.70 (2H, bt), 1.40 (4H, m) and 0.93 (3H, vt).

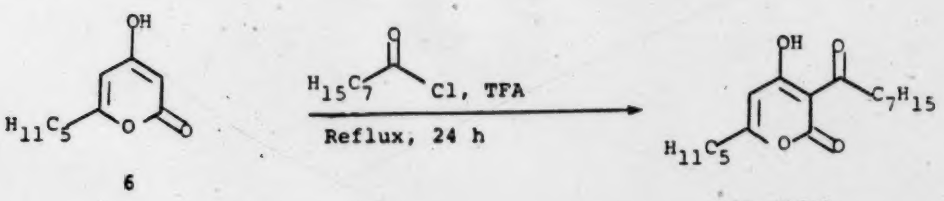


This dehydroacetic acid analogue **39**, was successfuly hydrogenated to the lachnelluloic acid analogue **40** by treatment with 10% Pd-C and 60 psi hydrogen in ethyl acetate.



The ^1H NMR of compound **40** is consistent with the structural assignment: δ 17.7 (1H, s, enolic OH), 4.55 (1H, ddq), 3.05 (2H, 2 x ddd), 2.68 (2H, 2 x dd), 1.70 (2H, m), 1.48 (3H, d), 1.38 (4H, m) and 0.92 (3H, vt).

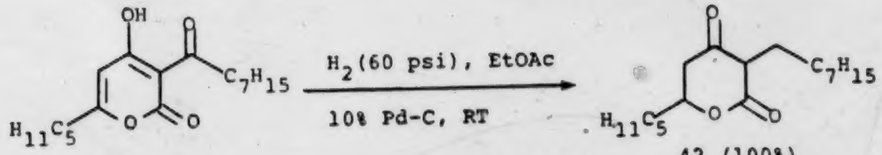
The same synthetic sequence was applied to the lactone **6**. Acylation of **6** with octanoyl chloride in refluxing TFA produced an oily product which after flash chromatography afforded, in 94% yield, dehydrolachnelluloic acid (**41**) as a pale yellow crystalline compound (MW 308 ($C_{18}H_{28}O_4$), IR 1725, 1635, 1605, 1560 cm^{-1}, ^1H NMR δ 16.8 (1H, s), 5.9 (1H, s)).



6

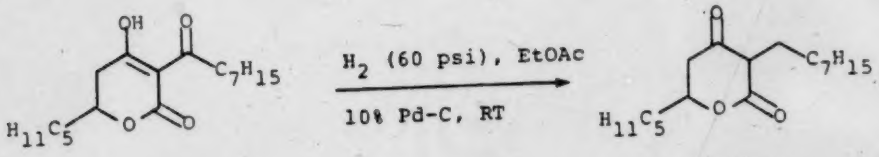
41 (94%)

Surprisingly, when dehydrolachnelluloic acid **41** dissolved in ethyl acetate was treated with hydrogen and 10% Pd-C (from a new bottle), lachnelluloic acid was not obtained. Instead, a white solid, which was identified as the β-ketolactone **42**, was obtained in quantitative yield. The ^1H NMR of compound **42** shows two independent spin systems and the absence of an enolic hydroxyl. The first spin system contains a D_2O exchangeable proton at δ 3.41 (1H, t, -CO-CH-CO-) coupled to a methylene that is part of an aliphatic chain at δ 1.92 (2H, bt). The second spin system is similar to the one present in lachnelluloic acid (see Chapter I, page 18), and contains a low field methylene at δ 2.73 and δ 2.42 (2H, 2 x dd) coupled to a carbinolic proton at δ 4.68 (1H, m).


OH
C_7H_{15}
$H_{11}C_5$
O
H_2(60 psi), EtOAc
10% Pd-C, RT

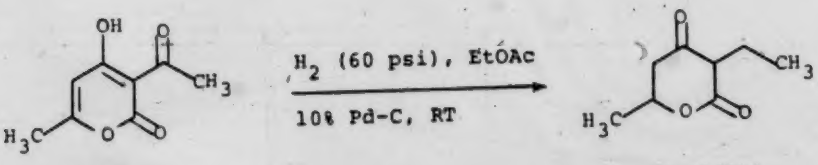
41 42 (100%)

As additional proof of its assigned structure, lachnelluloic acid was converted to the β-ketolactone **42** by hydrogenation.


OH
C_7H_{15}
$H_{11}C_5$
O
H_2 (60 psi), EtOAc
10% Pd-C, RT

1 42 (100%)

When the new batch of 10% Pd-C was tested using dehydroacetic acid (**9**) as substrate, the corresponding β-ketolactone **43** was obtained in quantitative yield.

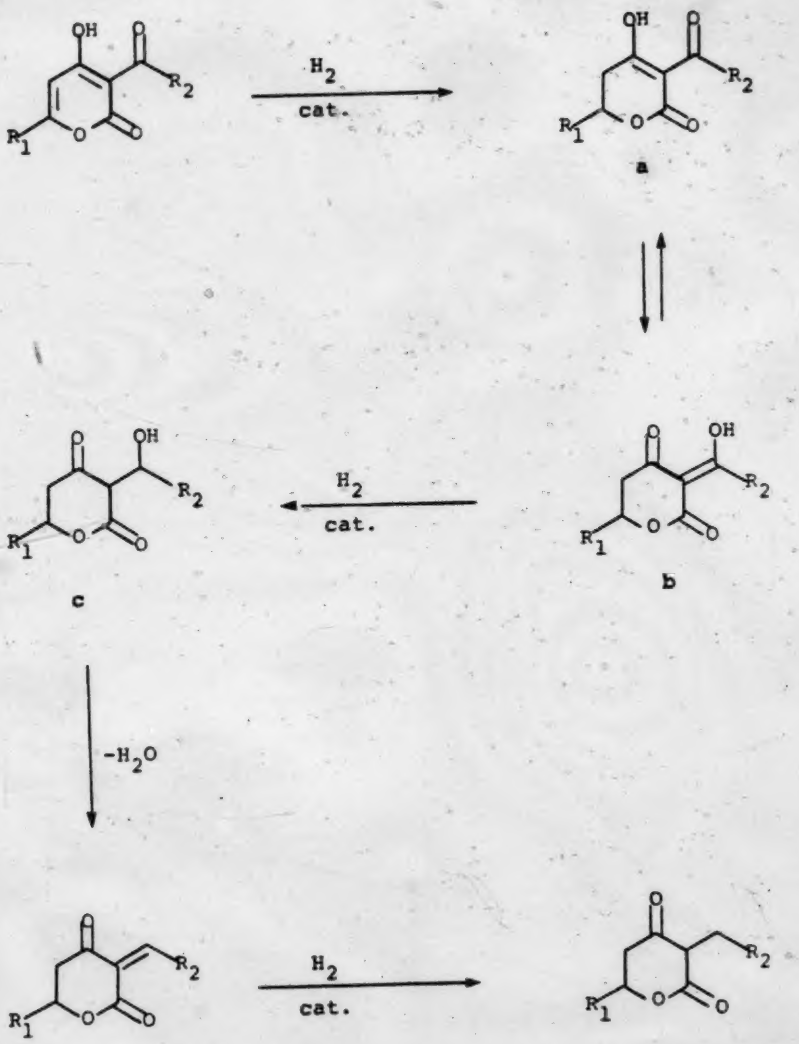

OH
CH_3
H_3C
O
H_2 (60 psi), EtOAc
10% Pd-C, RT

9 43 (100%)

Crystalline β-ketolactone **43** (mp 138-139°C) has a strong spicy odor. Its ^1H NMR spectra display a signal at δ 3.32 (1H, t) that is exchangeable with D_2O and which corresponds to the proton in the α-position to both carbonyls. β-Ketolactone **43** exists mostly as its diketo form, as shown by a negative ferric chloride test, the presence of a triplet at δ 3.32 in the ^1H NMR, the absence of the absorption for an enolic -OH and the presence of two characteristic carbonyl bands in the IR spectra (1760 cm^{-1} (lactone) and 1720 cm^{-1} (ketone)).

We believe that both β-ketolactones **42** and **43** are formed as shown in the following scheme. Hydrogenation of the double bond affords the dihydro-derivative, which exists in enolic forms **a** and **b**. The least hindered, and/or more reactive, form **b** is further hydrogenated to the β-hydroxyalcohol **c**. Dehydration of **c** followed by hydrogenation affords the β-ketolactone.

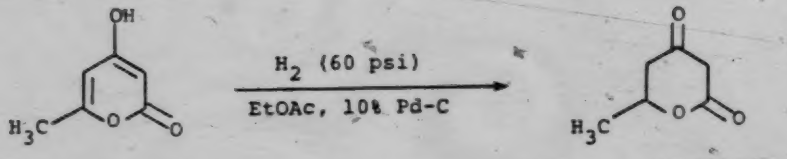
SCHEME 5:



OH
R_2
R_1
H_2
cat.
a
b
c
$-H_2O$

Enol form **a** probably hydrogenates very slowly relative to enol **b.**

Hydrogenation of triacetic acid lactone (**5**) under these same conditions gives β-ketolactone **44.**



5

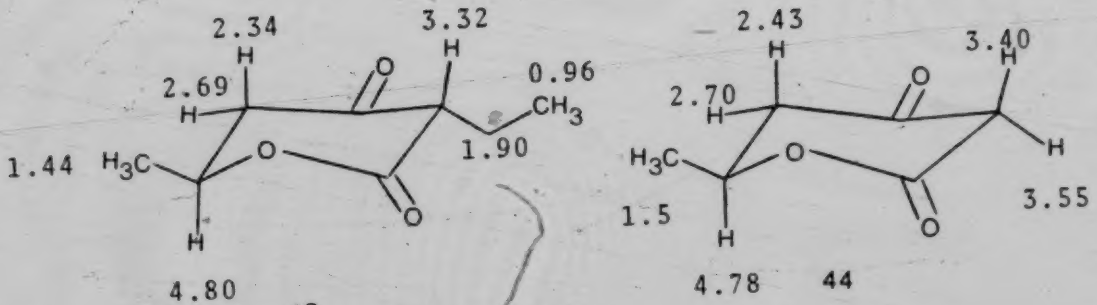
44 (100%)

As is the case for β-ketolactone **43**, compound **44** does not give a positive ferric chloride test and shows two D_2O exchangeable protons that are in α-position to two carbonyls at δ 3.55 (1H, d) and δ 3.40 (1H, d) in its ^1H NMR spectrum.

Conformational analysis of β-ketolactone **43** indicates that the methyl and ethyl substiuents exist in quasi equatorial positions, therefore the proton α to both carbonyls is quasi axial (δ 3.32). Of the two methylene protons on C-5 of compound **43**, the quasi axial proton (δ 2.34, J_{aa} = 12 Hz) appears at higher field than the quasi equatorial proton (δ 2.69, J_{ac} = 3 Hz).

In the ^1H NMR spectrum of β-ketolactone **44** a similar result is observed for the C-5 methylene protons: the quasi axial proton appears at higher field (δ 2.43) compared to the quasi equatorial proton (δ 2.70). In this β-ketolactone, the quasi axial proton at C-3 appears at δ 3.40 (d) and the quasi equatorial proton at δ 3.55 (d). These assignments are summarized in Scheme 6.

SCHEME 6:

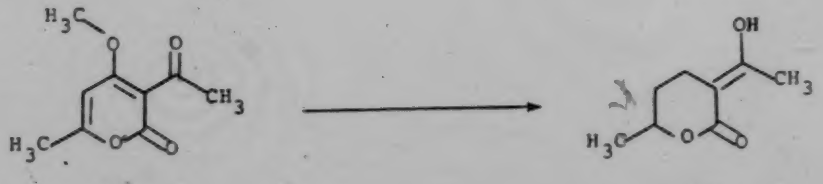


43

44

In both compounds, the quasi axial protons at C-3 and C-5 appear at higher field than the respective quasi equatorial protons. This is due to the anisotropic effect of the carbonyls on the quasi equatorial protons, which lie on a plane close to the plane of the carbonyls. Therefore, it seems clear that these β-ketolactones (**42** to **44**) are mainly unenolized in solution.

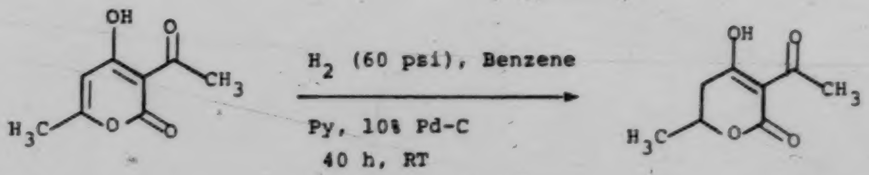
A somewhat similar reaction was reported for 3-acetyl-4-methoxy-6-methyl-2-pyrone (**45**), which under hydrogenation affords the enolized β-ketolactone **46**.[19]



45

46

In order to find a set of hydrogenation conditions which convert dehydrolachnelluloic acid (**41**) into lachnelluloic acid (1) using the new batch of 10% Pd-C catalyst, a series of experiments were carried out with dehydroacetic acid as the model substrate. The solvent, the catalyst, the time of reaction and the catalyst moderator (if any) were varied. The use of pyridine as moderator, and a reaction time of 40 h gave an almost quantitative yield of the desired product 15.



9 **15** (~95%)

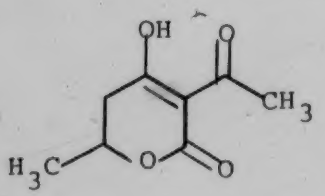
The results of some of these tests are summarized in the following table.

The ratio of products in the hydrogenation mixtures was determined by ^{1}H NMR spectroscopy, comparing the integration intensities of characteristic signals for each of the three compounds, dehydroacetic acid (9), β-ketolactone 43, and dihydrodehydroacetic acid (15). The quantity of the starting material 9 was determined by monitoring the signal for its enolic hydroxyl (δ 16.7); the desired product 15 by monitoring the signal at δ 17.9, which corresponds to its very characteristic enolic hydroxyl; and finally, the

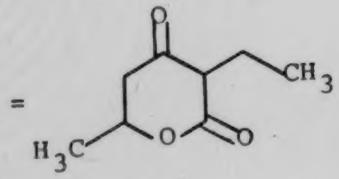
TABLE I. CATALYTIC HYDROGENATION OF DEHYDROACETIC ACID

CATALYST	SOLVENT	PRESSURE (psi)	TIME (hrs)	MODERATOR	SM	DP	UP
5% Pd-C	EtOAc	60	24	none	0.0	0.0	100.0
5% Pd-C	EtOAc	10	24	none	~ 50.0	0.0	~ 50.0
5% Pd-C	Ether	60	24	none	0.0	0.0	100.0
5% Pd-C	Ether	10	24	none	~ 75.0	0.0	25.0
5% Pd-C	EtOAc	60	24	pyridine	some	0.0	100.0
5% Pd-C	EtOAc	30	24	pyridine	50.0	0.0	50.0
5% Pd-C	EtOAc	60	24	quinoline	some	0.0	100.0
10% Pd-C	EtOAc	60	24	none	0.0	0.0	100.0
10% Pd-C	benzene	60	24	pyridine	60.0	40.0	trace
10% Pd-C	benzene	60	35	pyridine	10.0	80.0	trace
10% Pd-C	benzene	60	40	pyridine	trace	~ 95.0	trace
10% Pd-C	benzene	60	45	pyridine	0.0	90.0	10.0
10% Pd-C	benzene	60	60	pyridine	0.0	80.0	20.0

DP = DESIRED PRODUCT =

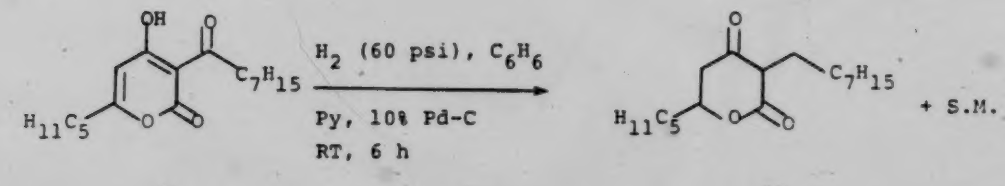


UP = UNDESIRED PRODUCT =



byproduct **43** was determined by monitoring the intensity of the signal at δ 3.32.

To our disappointment, when the new set of hydrogenation conditions was applied to the hydrogenation of dehydrolachnelluloic acid (**41**) only starting material and undesired β-ketolactone **42** were detected in the reaction mixture.

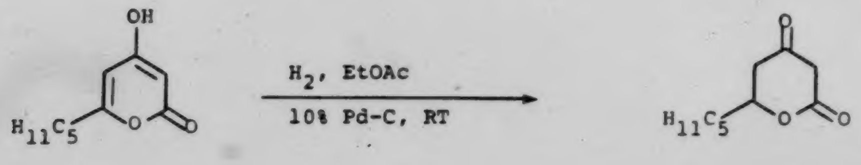


41 **42**

Shortened reaction times gave a higher percentage of starting material and a smaller amount of β-ketolactone. Disappointingly not even traces of lachnelluloic acid was detected. Clearly lachnelluloic acid is more reactive toward hydrogenation than dehydrolachnelluloic acid, and as soon as it is formed it is converted to the undesired compound **42**.

At this point a small change in the synthetic strategy was made; the reaction sequence was reversed. The pyrone **6** was first hydrogenated to the corresponding β-ketolactone, which was then acylated.

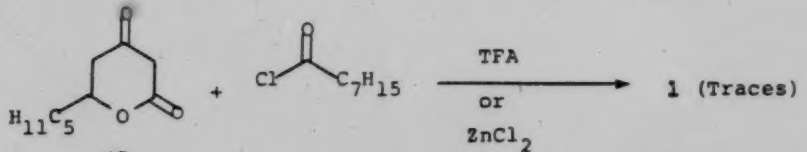
Pyrone **6** in ethyl acetate was easily hydrogenated over 10% Pd-C to β-ketolactone **47**, which was isolated in a very pure form simply by filtration of the reaction mixture and evaporation of the solvent (MW 184 ($C_{10}H_{16}O_3$), IR (cast) 1700, 1595 cm^{-1}, ^1H NMR δ 4.62 (1H), 3.56 (1H), 3.41 (1H).



6

47 (100%)

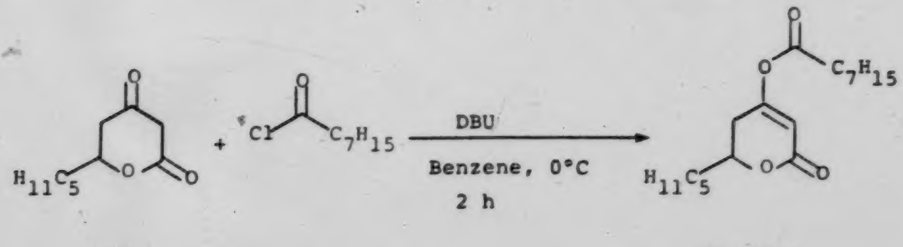
When direct C-acylation of the β-ketolactone **47** was attempted using acid catalysts (TFA, $ZnCl_2$) only very low yields of acylated product were detected.



47

This difficulty was overcome by using Tanabe's procedure: initial O-acylation followed by a base-catalyzed Fries-like rearrangement.[20]

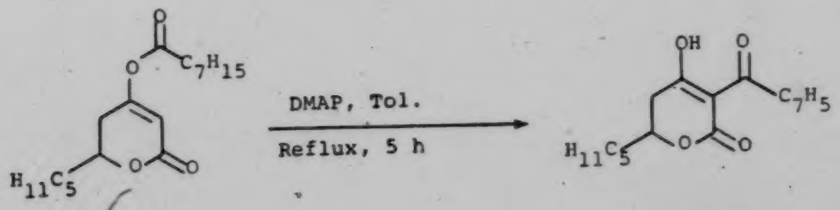
When β-ketolactone **47** was treated with DBU and octanoyl chloride in benzene at 0°C for 2 h, an oil was obtained in 91% yield. This oil was not fully characterized, but a ^1H NMR spectrum of its shows the presence of a vinyl proton at δ 5.82, as well as two virtually coupled methyl groups, as would be expected from the enol ester **48**.


H$_{11}$C$_5$
Cl
C$_7$H$_{15}$
DBU
Benzene, 0°C
2 h
C$_7$H$_{15}$
H$_{11}$C$_5$

47 **48** (91%)

This enol ester is very sensitive to hydrolysis, therefore it was not further purified.

The base catalyzed rearrangement of **48** was accomplished by treatment with a catalytic amount of 4-dimethylamino-pyridine (DMAP) in refluxing toluene for 5 h. Flash chromatography of the reaction mixture afforded pure lachnelluloic acid (**1**) in 75% yield (34.5% overall).


C$_7$H$_{15}$
H$_{11}$C$_5$
DMAP, Tol.
Reflux, 5 h
OH
C$_7$H$_5$
H$_{11}$C$_5$

48 **1** (75%)

The synthetic lachnelluloic acid was identical in all respects (IR, NMR, MS, UV) with the natural product.

The biological activity of the metabolites of *Lachnellula fuscosanguinea*.

The biological activity of the fungus *Lachnellula fuscosanguinea* was discovered by mycologists at the Canadian Forestry Service. This fungus, which in nature is strongly antagonist to phytopathological fungi such as *Fomes pini* and *Ceratocystis ulmi*, was shown to possess antifungal activity *in vitro*.

Two different bioassays were used to test for antifungal activity of the crude extracts or pure compounds. In method A, a petri dish is inoculated with a plug of mycelium of the fungus being tested. The compound or mixture of compounds to be studied are dissolved in known concentration and absorbed on a sterile filter paper disk (1 cm diameter). After the solvent evaporates from the disk, the disk is applied to the inoculated petri dish 1 to 2 inches from the plug of mycelium. Growth of the wood-rotting test fungus is slow, thus the test results are recorded between 3 weeks and 2 months after inoculation. A result is considered positive when the fungal mycelium does not grow in the area of the impregnated paper disk, if the fungus overgrows the disk the result is recorded as negative.

In method B, which is a modification of the Kirby-Bauer bioassay, the petri dish is swabbed with an inoculum prepared by growing the test fungus in sterile liquid media (Muller-Hinton or PDB). The liquid inoculum is spread on the whole surface of the agar plate using a sterile cotton swab. The disk impregnated with the compounds being tested is immediately applied to the petri dish. The results of this test can be recorded two to four days after inoculation, depending on the rate of fungal growth. A positive result is recorded when the fungal mycelium grows over the petri dish except in a zone surrounding the impregnated disk. If a solvent blank and standard concentrations of test compounds are used, relative antifungal activities can be obtained by measuring the inhibition zone diameter.

For testing compounds or mixtures of compounds against bacteria, method B was used, i.e., a liquid inoculum of bacteria was used.

The broth and mycelium extracts of cultures of Lachnellula fuscosanguinea were tested against a variety of bacteria and fungi. These results for 5% solutions of the crude extracts are shown in Table II. The results of the antibacterial tests were all negative.

Table II Bioactivity of crude extracts of Lachnellula fuscosanguinea against wood rotting fungi.

Fungi	Broth Extract	Mycelium Extract
Fomes pini	+	-
Polyporus betulinus	+	-
Coneophora putena	-	-
Phoma sp.	+	—
Pleurotos ostreatus	-	-
Ceratocystis ulmi	+	+

On one occasion the crude extract from the fermentor was active against Fomes pini, Polyporus betulinus, Phoma sp. and Ceratocystis ulmi, but these results could not be reproduced. Generally, the crude extracts from the fermentor are inactive against the bacteria and fungi tested.

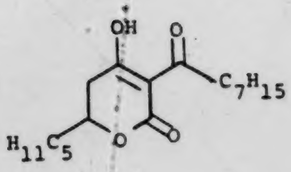
Among the metabolites isolated from L. fuscosanguinea only lachnelluloic acid and lachnellulone show biological activity. Lachnellulone is by far the most potent of the two compounds.

Several lachnelluloic acid analogues have been synthesized. The bioactivity of these compounds against pathogenic fungi have been compared to the biological activity of the two active natural products and the results are summarized in Table III. The two fungi used were C. ulmi (the causative agent of Dutch elm disease) and H. mammatum (produces canker in poplar).

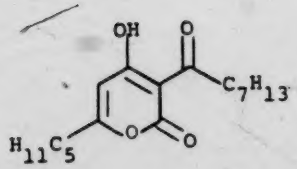
Table III. Antifungal activity of lachnellulone, lachnelluloic acid and analogues.

Compound*	Activity Against Ceratocystis ulmi (Diameter in cm)	Activity Against Hypoxylon mammatum (Diameter in cm)
Lachnellulone (**49**)	7.6	7.0
Lachnelluloic acid (**1**)	3.0	0.0
Dehydrolachnelluloic acid (**14**)	3.0	0.5
4-Hydroxy-6-methyl 3-octyl-2-pyrone (**39**)	7.0	7.5
5-Hexyl-4-hydroxy-6-pentyl-2-pyrone (**37**)	0	0
Compound (**38**)	0	3.0

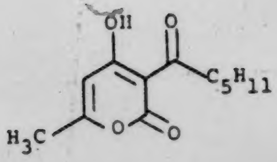
*The concentration used was 0.1%.



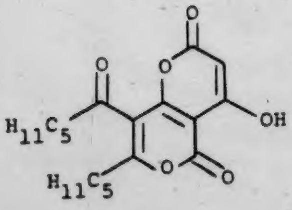
1



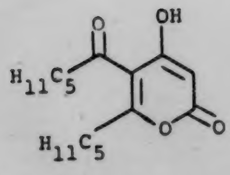
14



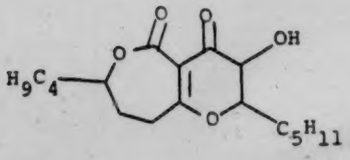
39



38

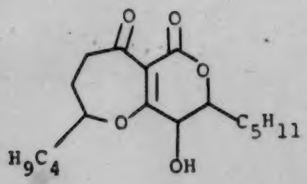


37



or

49



As shown in Table III lachnellulone is the strongest antifungal compound of this series that has been tested so far.

Interestingly, in the series of lachnelluloic acid and analogues, lachnelluloic acid is not the most potent bioactive agent. When comparing the activity of compounds **37** to **38** it is interesting to note that substitution at C-3 in the pyrone ring seems necessary for the pharmacological activity. Thus, compound **37**, in which the C-3 position is unsubstituted, does not show bioactivity, while all the other compounds show bioactivity to some degree. The bicyclic compound **38**, which is derived from **37** by substitution at C-3, also displays antifungal properties against H. mammatum.

Dehydrolachnelluloic acid is a slightly more general fungicide than lachnelluloic acid, a fact that supports the supposition that the active form of lachnelluloic acid is its 5,6-dehydro form. It is possible that lachnelluloic acid, which we believe to be an intermediate in the biosynthesis of lachnellulone, is oxidized in vivo to a more potent antifungal form.

The size of the chain at C-6 of the pyrone seems to play a role in the bioactivity of the structural types. Decreasing the chain length to one carbon substantially increases the antifungal properties of the pyrone. This enhancement in bioactivity is large enough to make compound **39** almost as bioactive as lachnellulone. These trends have been observed using Fomes igniarus (decay fungus of poplar), Polyporus tormentosum (root rot of conifer) and Fomes pini (decay fungus of conifer).

The relative bactericidal activity of lachnellulone, lachnelluloic acid and the synthetic analogues was studied using the Kirby-Bauer test (method B) for 0.1% solutions of the compounds. The results are shown in Table IV.

Many more studies will be required to understand the structure-activity relationships of this new group of fungicides in order to prove or disprove the general trends observed.

Undoubtedly, lachnellulone is a strong inhibitor of C. ulmi and H. mammatum. We believe that it is the compound mainly responsible for the antifungal properties of L. fuscosanguinea.

Table IV. Kirby-Bauer test for lachnellulone, lachnelluloic acid and the synthetic analogues.

List of cultures	Tested Material*					
	49	1	14	39	37	38
Enterobacter cloacae	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-	-
Proteus vulgaris	-	-	-	13	-	-
Pseudomonas aeruginosa	9	17	11	-	21	-
Salmonella typhimurium	-	-	-	-	-	-
Serratia marscens	-	-	-	-	-	-
Staphylococcus aureus	10	13	9	9	15	9
Staphylococcus epidermis	8	23	14	13	20	9
Streptococcus pyrogenes	9	15	-	9	20	-
Candida albicans	-	-	-	13	-	-

*The activity is reported as the diameter of the inhibition zone in mm.

Experimental

Trans-2-octenoyl chloride (**20**):

Hexanal (0.05 moles) was mixed with malonic acid (0.05 moles) and pyridine (24 mL) in a round bottom flask with a reflux condenser. The mixture was stirred constantly and warmed in a water bath for 2 h. The heterogeneous mixture produced a gas (CO_2) when heated and slowly became a colorless solution. This solution was diluted with ether (50 mL) and treated with cold 1:1 HCl (100 mL). The two layers were separated and the aqueous layer was extracted once with ether (50 mL). The combined ether extracts were dried over anhydrous Na_2SO_4, filtered and concentrated to afford a clear liquid, which was distilled under reduced pressure (130°C at 12 torr) to give 4.5 g (63%) of a mixture of trans and cis-2-octenoic acid. IR (cast) 3300, 1960, 1920, 1690, 1650, 1460, 1420 and 1285 cm^{-1}. ^1H NMR δ 11.4 (1H, bs), 7.1 and 6.4 (1H, dt, t), 5.8 (1H, bd), 2.3 (2H, m), 1.4 (6H, m), 0.9 (3H, vt).

Commercial trans-2-octenoic acid (24 mmoles) was slowly added to freshly distilled thionyl chloride (50 mmoles) in a round bottom flask with a reflux condenser and a $CaCl_2$ drying tube. The mixture was kept at 50°C for 45 min until the solution became dark brown. The excess of thionyl chloride was eliminated in the rotatory evaporator and the resulting liquid residue was distilled under reduced pressure (70°C, 1.5 torr) to afford pure trans-2-octenoyl chloride, 2.50 g (65%). IR (cast) 2960, 2930, 2860, 1750, 1625, 1460, 1130, 1100, 1020, 980, 960, 770 and 610 cm^{-1}. ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.2 (1H, dt), 6.0 (1H, dm), 2.3 (2H, bt), 1.4 (6H, bs) and 0.90 (3H, vt).

Ethyl 3-oxodecanoate (21):

2-Nonanone (0.3 moles) was dissolved in diethyl carbonate (150 mL) in a two neck round bottom flask with a dropping funnel and a fractionation column connected to a water condenser in a distilling position. The mixture of ketone and carbonate was heated to reflux. To this boiling solution, sodium ethoxide (0.4 moles) in ethanol, was slowly added and at the same time ethanol was being distilled out constantly from the reaction mixture. When no more ethanol was being distilled out, the viscous reaction mixture was poured over a 1:1 mixture of 15% HCl and ice (400 mL). The organic layer was separated and washed twice with distilled water and once with brine. After drying with anhydrous Na_2SO_4 and filtering, the diethyl carbonate was eliminated from the reaction mixture in the rotatory evaporator, the resulting liquid residue was distilled under reduced pressure (2.0 torr) and the fraction that distilled at 160-170°C was dissolved in 95% ethanol (100 mL) and treated with saturated copper chloride (50 mL). The green precipitate formed was filtered by suction and washed with cold 95% ethanol. The washed crystals of copper complex were dissolved in chloroform (75 mL) and mixed with 5% HCl (100 mL) for 1 h. The clear layer of chloroform was separated and washed with distilled water and brine, to afford after evaporation of the solvent pure ethyl 3-oxodecanoate **21** (50%).

IR (cast) 2950, 2920, 2850, 1740, 1715, 1640, 1460, 1370, 1260, 1150, 1035 and 790 cm^{-1}.

HREIMS: m/z 214 ($C_{12}H_{22}O_3$). ^1H NMR (60 MHz, $CDCl_3$): δ 4.3 (2H, q), 3.4 (2H, s), 2.5 (2H, bt), 1.7-1.1 (14H, m), 0.90 (3H, vt).

9-Carboethoxy-8,10-dioxo-trans-6-decaheptene (**23**).

Dry magnesium metal (12 matg) and dry ethanol (1.5 mL) with few drops of CCl_4 were mixed in a 25 mL three neck round bottom flask connected to a dropping funnel, a reflux condenser with a calcium chloride drying tube and a nitrogen inlet. The mixture was warmed to 40°C to start the reaction and left for 1 h. Dry benzene (3.0 mL) was added to the mixture, which was left to react for one more hour. Ethyl

3-oxodecanoate (**21**, 10 mmoles) was added dropwise to the reaction mixture. After stirring for 8 h all the magnesium had been dissolved and the resulting green solution was cooled to 0°C in an ice-water bath. To this cold solution, trans-2-octenoyl-chloride (11 mmoles) was added dropwise and with constant stirring. This solution was let to warm to room temperature and kept constantly stirred for 8 h. The reaction mixture was poured over a mixture of ice (5 g) and concentrated H_2SO_4 (0.5 mL). The organic layer was separated and the aqueous phase extracted with ether. The combined organic extracts were washed once with 10% H_2SO_4, 5% $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered and concentrated to give as a single product compound **23** in almost quantitative yield (3.6 g).

TLC: Rf 0.5 in Skellysolve B:Chloroform (1:1).

IR (cast): 2950, 2920, 2860, 1705, 1680, 1640 and 1075 cm^{-1}.

^1H NMR (80 MHz, $CDCl_3$): δ 17.4 (1H, s), 7.0 (1H, m), 6.4 (1H, bd), 4.2 (2H, q), 2.6 (2H, m), 2.2 (2H, m), 1.8 (2H, m), 1.4 (18H, m), 0.9 (6H, vt).

3-Carboethoxy -2-heptyl-6-pentyl-5,6-dihydro-4-pyrone (**24**).

9-Carboethoxy -8,10-dioxo-trans-6-decaheptene (**23**, 0.6 mmoles) was dissolved in trifluoroacetic acid (TFA, 20 mL) and heated to reflux for 2 h. The TFA was evaporated and the residue was separated in a flash column (30 mm diameter column, Skellysolve B:ethyl acetate:acetic acid:8:2:trace). Fractions 10 to 15 contained the pure desired product (**24**); 138 mg (69% yield).

IR (cast): 2950, 2920, 2850, 1710, 1670, 1585, 1445, 1180, 1135 and 1070 cm^{-1}.

HREIMS: m/z 338 ($C_{20}H_{34}O_4$, 100%, M^+), 293 ($C_{18}H_{29}O_3$, 72, M-C_2H_5O), 267 ($C_{15}H_{24}O_4$, 83, M-C_5H_{10}), 265 ($C_{17}H_{29}O_2$), 241 ($C_{13}H_{21}O_4$), 221 ($C_{13}H_{17}O_3$).

^1H NMR (200 MHz, $CDCl_3$): δ 4.40 (1H, m, coupled to δ 2.50, 1.84 and 1.65), 4.30 (2H, q), 2.50 (2H, dd, coupled to δ 4.40 and 1.65), 1.84 (1H, m), 1.65 (1H, m), 1.33 (18H, bm), 0.93 (6H, vt).

Base-catalyzed rearrangement of the dihydropyrone (**24**).

The following sets of conditions were tried unsuccessfully for the base-catalyzed rearrangement of the dihyropyrone **24**:

1. Dihydropypone (**24**, 8 mg) was mixed with 1 M NaOH (2.0 mL) and stirred at room temperature overnight. The reaction mixture was then acidified with concentrated HCl to pH 1 and extracted with ether. The ether extracts were dried over anhydrous Na_2SO_4 and filtered. TLC analysis of

this extract did not reveal the presence of lachnelluloic acid, but the presence of starting material **24** and its acyclic form **23.**

This experiment was repeated using reaction times of 1 h and 3 h with the same negative results.

2. The procedure described above was repeated using 1 M NaOH in 95% EtOH instead of aqueous 1 M NaOH. The same negative results were obtained.

4-Methoxy-6-methyl-2-pyrone (**25**) and 2-methoxy-6-methyl-4-pyrone (**26**).

Triacetic acid lactone (49 mmoles) was suspended in acetone (100 mL) and treated with excess of 1 M ethereal diazomethane at 0°C with constant stirring. The reaction mixture was allowed to reach room temperature and evaporation of the solvent left a yellowish solid residue. Flash chromatography of this residue (50 mm diameter column, benzene:acetone:3:1) afforded two pure compounds. Fractions 6 to 10 from the flash column gave 4-methoxy-6-methyl-2-pyrone (**25**) as white crystals (70%). Melting point 80-80.5°C.

TLC: Rf 0.51 in benzene:acetone:3:1.

UV (MeOH): λ_{max} (ε) 280 (23500), 210 (21000).

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (1H, bd), 5.38 (1H, d), 3.77 (3H, s), 2.18 (3H, s).

Fractions 33 to 50 from the flash column afforded 2-methoxy-6-methyl-4-pyrone (**26**) as white crystals (mp 86-87°C) in 30% yield.

TLC: RF 0.35 in 2% methanol in chloroform.

UV (MeOH), λ_{max} (ε) 245 (28700)

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.04 (1H, bq), 5.52 (1H, d), 3.90 (3H, s) and 2.28 (3H, s).

Reaction of 2-methoxy-6-methyl-4-pyrone (**26**) with nBuLi.

Pyrone **26** (1 mmole) was dissolved in dry THF (2.0 mL) and the solution was kept under a nitrogen atmosphere while being cooled to -78°C with a dry ice-acetone bath. To the cold solution 1.48 M nBuLi (1 mmol) in benzene was added slowly and with constant stirring to produce a deep red solution. When this solution was treated with excess of methyl iodide (5 mmole) in dry THF (5 mL) and the reaction mixture brought to room temperature the red solution did not decolorize. TLC analysis of this reaction mixture showed several products present in comparable amounts.

This reaction was repeated using LDA (~ 2 equivalents) with the same results. When D_2O was used instead of methyl lithium during the work up the reaction mixture remained colored and complex by TLC.

Ethyl 3-oxooctanoate (**28**)

This compound was prepared using the same procedure used for ethyl 3-oxodecanoate (**21**) (see page 172). 2-Heptanone was the methyl ketone employed (0.35 moles) in diethyl carbonate (200 mL). Purification of the impure β-ketoester by complexation with copper acetate gave **28** as a colorless liquid (bp 128-129°C at 12 torr).

TLC: Rf 0.54 in Skellysolve B:EtOAc:96:4.

IR (neat): 2960, 2930, 2870, 1740, 1715, 1640, 1460, 1410, 1370, 1310, ~~1235,~~ 1155, 1030 and 740 cm^{-1}.

HREIMS: m/z 186 ($C_{10}H_{18}O_3$, 5%).

^{1}H NMR (80 MHz, $CDCl_3$): δ 4.20 (2H, q), 3.45 (2H, s), 2.60 (2H, t), 1.40 (9H, m), 0.90 (3H, vt).

3-Hexanoyl-4-hydroxy-6-pentyl-2-pyrone (**29**).

Ethyl 3-oxooctanoate (**28**, 12 mmoles) was mixed with $NaHCO_3$ (2 mg) in a round bottom flask with a fractionation column connected to a condenser in distillation position. The flask was kept in an oil bath at 200°C for 14 h. The dark reaction mixture was purified using a flash column (50 mm diameter column, Skellysolve B:EtOAc:100:4). The product was obtained impure in fractions 16 to 19. Recrystallization from Skellysolve B afforded white crystals of the desired material (0.3 g, 20%).

TLC: Rf. 0.8 1% MeOH in $CHCl_3$.

HREIMS: m/z 280 ($C_{16}H_{24}O_4$, M^+), 262 ($C_{16}H_{22}O_3$), 237 ($C_{13}H_{17}O_4$), 224 ($C_{12}H_{16}O_4$, 100%), 209 ($C_{11}H_{13}O_4$), 168 ($C_8H_8O_4$), 153 ($C_7H_{15}O_4$), 141($C_8H_{13}O_2$) and 99 ($C_6H_{11}O$).

^{1}H NMR (80 MHz, $CDCl_3$): δ 16.8 (1H, s), 5.9 (1H, s), 3.2 (2H, t), 2.6 (3H, dt), 1.6 (4H, bt), 1.3 (12H, m) and 0.9 (6H, vt).

2,4-Nonanedione (32)

Sodium amide (0.5 moles) was suspended in dry THF (50 mL). To this suspension, 2-heptanone (0.25 moles) in THF (25 mL) was added for a period of 10 minutes. The resulting mixture was vigorously stirred for 5 minutes before adding the methyl acetate (1 mole). The slow addition of methyl acetate produced a vigorous reaction, making it necessary to cool the reaction flask in an ice-water bath. Once the addition of methyl acetate was completed, the viscous reaction mixture was heated to reflux for one hour. After cooling to room temperature the reaction mixture was treated with 10% HCl until an acid reaction with litmus paper was obtained. The acidified reaction mixture was then diluted with distilled water (100 mL) and extracted with ether. The combined ether extracts were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford a red liquid residue. This residue was dissolved in methanol (100 mL) and mixed with a hot solution of copper acetate (20 g), in water (150 mL). The green precipitate formed was separated by filtration and washed with Skellysolve B. This copper complex was dissolved in ether (200 mL) and treated with 10% H_2SO_4 (200 mL) under vigorous stirring. After 10 minutes the originally green organic layer had become yellow and the lower aqueous layer had become blue. The ethereal solution was separated, washed with distilled water, dried over anhydrous Na_2SO_4, filtered and concentrated to give a brown liquid which was shown to be a mixture of compounds by TLC. Vacuum distillation of this brown residue (1.8 torr) afforded pure 2,4-nonanedione as a colorless liquid, bp 60-62°C at 1.8 torr, n_D^{20} 1.4584 (53% yield).

TLC: Rf 0.6 in Skellysolve B:ethyl acetate:9:1.

IR (cast): 2960, 2935, 2870, 1728, 1708, 1615, 1460, 1420, 1360, 1245 and 780 cm^{-1}

^1H NMR (80 MHz, $CDCl_3$): δ 5.4 (1H, s), 3.5 (0.2H, s), 2.25 (2H, t), 2.0 (3H, s), 1.6 (2H, bt), 1.4 (4H, m), 0.90 (3H, vt).

Reaction of 2,4-nonanedione (**32**) with malonyl dichloride:

In a 5 mL round bottom flask, equal volumes of malonyl dichloride (1 mL) and 2,4-nonanedione (**32**) were mixed. This mixture was warmed in a water bath at 70°C for 10 minutes. When the evolution of hydrogen chloride from the reaction flask had stopped, the reaction mixture had turned red. Flash chromatography of the crude reaction mixture (50 mm diameter column, 2% MeOH in $CHCl_3$) afforded in fractions 11-20 a white crystalline compound (mp 119-121°C) which was shown to be the bicyclic pyrone **33** (or a mixture of regioisomers) (30% yield).

TLC: Rf 0.35 in 2% MeOH in $CHCl_3$.

HREIMS: m/z (formula, intensity, fragment) 292 ($C_{15}H_{16}O_6$, 37, M$^+$), 277 ($C_{14}H_{13}O_6$, 10, M-CH_3, 264 ($C_{14}H_{16}O_5$, 9, M-CO), 249 ($C_{12}H_9O_6$, 7, M-C_3H_7), 246 ($C_{14}H_{14}O_4$, 24, M-CH_2O_2), 236 ($C_{11}H_8O_6$, 85, M-C_4H_8), 221.1 (100%), 221.0 ($C_{10}H_5O_6$, 16, M-C_5H_{11}), 208 ($C_{10}H_8O_5$, 59, M-C_5H_8O), 194 ($C_9H_6O_5$, 17).

^{1}H NMR (80 MHz, $CDCl_3$): δ 10.4 (1H, s), 5.6 (1H, s), 2.9 (1H, t, J = 6.5 Hz), 2.4 (3H, s), 1.7 (2H, bt, J = 6.5 Hz), 1.5 to 1.3 (4H, m), 0.90 (3H, vt).

A more polar compound (actually a mixture of two compounds) was found in fraction 22-35. This white amorphous solid (mp 100-102°C) was shown to be a mixture of the two pyrones **34** and **35** (30% yield).

TLC: Rf. 0.4 in 5% MeOH in $CHCl_3$

UV (MeOH): λ_{max} 229 (ε 6000), 270 (ε 3000) nm.

IR (cast): 3060, 2985, 2940, 2880, 2735-1620 (broad), 1550, 1460, 1265, 1000 and 740 cm^{-1}.

HREIMS: m/z (formula, intensity, fragment). 224 ($C_{12}H_{16}O_4$, 58, M^+), 209 ($C_{11}H_{13}O_4$, 9, M-CH_3), 196 ($C_{11}H_{16}O_3$, 52, M-CO), 181 ($C_9H_9O_4$, 21, M-C_3H_7), 178 ($C_{11}H_{14}O_2$, 15, M-CH_2O_2), 168 ($C_8H_8O_4$, 84, M-C_4H_8), 153 ($C_7H_5O_4$, 100, M-C_5H_{11}), 140 ($C_7H_7O_3$, 92, M-C_5H_8O), 125 ($C_6H_5O_3$, 32, M-$C_6H_{11}O$), 111 ($C_5H_3O_3$, 42, M-$C_7H_{13}O$), 98 ($C_5H_6O_2$, 16, M-$C_7H_{10}O_2$) and 85 ($C_4H_5O_2$, 18, M-$C_8H_{11}O_2$).

^1H NMR (80 MHz, $CDCl_3$): δ 11.2 (1H, bs), 5.5 (1H, s), 2.9 (2H, t, J = 7.0 Hz), 2.7 (3H, s), 1.8 (2H, m), 1.4 (4H, m) and 0.95 (3H, vt).

Deacylation of the pyrones 5-acetyl-4-hydroxy-6-penthyl-2-pyrone (**35**) and 5-hexanoyl-4-hydroxy-6-methyl-2-pyrone (**34**).

The mixture of acyl pyrones (8.0 mmoles) was dissolved in 90% H_2SO_4 (5 mL) and heated to 130°C with constant stirring for 30 min. After cooling to room temperature, the dark reaction mixture was diluted with water (60 mL) and extracted with chloroform (3 x 30 mL). The combined chloroform extracts were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to yield a dark residue composed of a mixture of crystal and an oil. Flash chromatography of this mixture (40 mm diameter column, 3.5% MeOH in $CHCl_3$) afforded two products. Fractions 11 to 14 contained 4-hydroxy-6-pentyl-2-pyrone (**6**, 13%). Mp 50-51°C. TLC: Rf 0.42 in 5% MeOH in $CHCl_3$.

^1H NMR (80 MHz, $CDCl_3$): δ 10.96 (1H, bs), 6.05 (1H, d), 5.60 (1H, d), 2.50 (2H, t), 1.60 (2H, bt), 1.3 (4H, m) and 0.90 (3H, vt).

Fractions 14 to 26 contained triacetic acid lactone (**5**), 400 mg (38%), mp. 179-180°C.

^1H NMR (60 MHz, DMSO-$\underline{d}_6$): δ 5.98 (1H, bm), 5.29 (1H, bd), 2.18 (3H, bs)

6,8-Tridecanedione (36)

Sodium amide (1 mole was suspended in dry THF (100 mL) in a 500 mL two neck round bottom flask to which a dropping funnel and a reflux condenser with a $CaCl_2$ drying tube had been adapted. 2-Heptanone (0.33 moles) in THF (50 mL) was added from the funnel in a period of 10 minutes. After stirring for 5 minutes, methyl hexanoate (70 mL) in THF 50 mL) was slowly added to the reaction flask. The viscous reaction mixture was heated under reflux for 1 hour and let to cool to room temperature. The cool reaction mixture was acidified with 10% HCl until all the precitiate had dissolved. The upper organic layer was separated, washed with distilled water, dried over anhydrous Na_2SO_4, filtered and concentrated to yield a red solution. Vacuum distillation (3 torr) produced a fraction (130°C) containing the desired β-diketone, which was further purified by complexation with copper acetate (~ 100 mL of saturated solution). The copper complex was separated by filtration and washed with 95% EtOH. The clean green crystals of complex were then dissolved in chloroform (100 mL) and decomposed with 10% H_2SO_4 (200 mL). The colorless chloroform solution afforded, after being dried and concentrated, pure 6,8-tridecanedione (**36**), 35 g (54%), n_D^{20} 1.442.

TLC: Rf 0.65 in Skellysolve B:ethyl acetate:9:1.

IR (cast): 2960, 2930, 2860, 1705 (w), 1610, 1460, 1150, 1110, 950 and 780 cm^{-1}.

HREIMS: m/z (formula, intensity) 212 ($C_{13}H_{24}O_2$, 11), 170 ($C_{10}H_{18}O_2$, 13), 156 ($C_9H_{16}O_2$, 34), 141 ($C_8H_{13}O_2$, 100), 100 ($C_5H_8O_2$, 50) and 99 ($C_6H_{11}O$, 77).

^1H NMR (80 MHz, $CDCl_3$): δ 15.40 (1H, bs), 5.50 (1H, s), 3.58 (fraction, s), 2.52 (fraction, t), 2.30 (4H, t), 1.62 (4H, bt), 1.30 (8H, m), 0.90 (6H, vt).

Condensation of 6,8-tridecanedione with malonyl dichloride.

6.8- Tridecanedione (24 mmoles) was set into a round bottom flask connected to a dropping funnel and a gas outlet. Malonyl dichloride (24 mmoles) was slowly added to the β-diketone from the dropping funnel, and the reaction mixture was heated in a water bath at 80°C. A copious amount of hydrogen chloride, which was being absorbed by a water aspirator, was produced in the darkening reaction mixture. When the evolution of HCl had stopped (30 minutes), the reaction mixture was dissolved in ether and washed with distilled water and finally with brine. The ether extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford a red-brown solid residue which was recrystallized from hexane:ether:1:1 several times to give pale yellow flakes of 5-hexanoyl-4-hydroxy-6-pentyl-2-pyrone (**37**) 3.9 g (58% yield), mp 91-92°C.

TLC: Rf 0.55 in 2% MeOH, 2% HOAc in $CHCl_3$.

FTIR (KBr): 3370, 2960, 2930, 2860, 2620, 2500, 1705, 1690, 1620, 1565, 1460, 1290, 985 and 830 cm^{-1}.

UV (MeOH) λ_{max} 225 (ε 6050); 261 (ε 5410), 288 (ε 5250) nm. Microanalysis: calculated C, 68.55; H, 8.63; O, 22.83. Found: C, 68.76, 68.92, H, 8.70, 8.69, O, 22.54, 22.89.

HREIMS: m/z (formula, intensity, fragment). 280 ($C_{16}H_{24}O_4$, 25, M^+), 252 ($C_{15}H_{24}O_3$, 22, M-CO), 237 ($C_{13}H_{17}O_4$, 15, M-C_3H_7), 224 ($C_{12}H_{16}O_4$, 18), 210 ($C_{11}H_{14}O_4$, 8), 209 ($C_{15}H_{13}O$, 100, M-C_5H_{11}), 206 ($C_{12}H_{14}O_3$, 27), 196 ($C_{11}H_{16}O_3$, 23), 181 ($C_9H_9O_4$, 14), 168 ($C_8H_8O_4$, 44), 153 ($C_7H_5O_4$, 26) and 99 ($C_6H_{11}O$, 67).

^{1}H NMR (200 MHz, $CDCl_3$): δ 16.71 (1H, bs), 5.56 (1H, s), 2.82 (2H, t, J = 7.5 Hz), 2.73 (2H, bt, J = 8.0 Hz), 1.70 (4H, m), 1.31 (8H, m) and 0.90 (6H, vt).

^{13}C NMR (50 MHz, $CDCl_3$): δ 202.6 (s), 169.8 (s), 169.2 (s), 165.1 (s), 115.4 (s), 90.1 (d), 44.4 (t), 32.5 (t), 31.3 (t), 31.2 (t), 27.4 (t), 23.8 (t), 22.3 (t), 22.1 (t), 13.8 (q) and 13.7 (q).

From the mother liquors of the recrystallized reaction mixture, a second product was isolated by flash chromatography (50 mm diameter column, chloroform:acetic acid:96:4). Fractions 9 to 24 from this chromatographic process gave an orange solid which was decolorized with activated charcoal and recrystallized from hexane-ether to give 5-hexanoyl-4-hydroxyl-6-pentyl-2-pyrone (**37**). Fractions 5 to 7 of this flash column chromatography gave a pale yellow solid which was recrystallized from hexane to afford white needlelike crystals of the bicyclic pyrone **38**, 0.8 g (10% yield), mp 80-82°C.

TLC: Rf 0.8 2% MeOH, 2% HOAc in $CHCl_3$.

FTIR ($CHCl_3$, cast): 3200 2950, 2920, 2860, 1705, 1695, 1635, 1555, 1435, 1305, 1235, 1155, 1015 and 750 cm^{-1}.

Microanalysis: Calculated for C, 65.50; H, 6.94; O, 27.55. Found: C, 65.57; H, 7.04; O, 27.39.

HREIMS: m/z (formula, intensity, fragment). 348 ($C_{19}H_{24}O_6$, 100, M^+), 320 ($C_{18}H_{24}O_5$, 16, M-CO), 305 ($C_{16}H_{17}O_6$, 20, M-C_3H_7), 277 ($C_{14}H_{13}O_6$, 62, M-C_5H_{11}), 263 ($C_{14}H_{15}O_5$, 21, M-C_5H_7O), 235 ($C_{12}H_{11}O_5$, 30, M-$C_6H_{13}O$).

^1H NMR (200 MHz, $CDCl_3$): δ 10.75 (1H, s), 5.64 (1H, s), 2.91 (2H, t, J = 7 Hz), 2.64 (2H, bd, J = 7 Hz), 1.72 (4H, m), 1.36 (8H, m) and 0.93 (6H, vt).

^{13}C NMR (50 MHz, $CDCl_3$): δ 197.6 (s), 169.3 (s), 165.5 (s), 163.7 (s), 161.9 (s), 158.7 (s), 114.9 (s), 95.0 (s), 90.2 (d), 44.4 (t), 32.0 (t), 30.9 (t), 30.8 (t), 27.1 (t), 23.2 (t), 21.8 (t), 13.4 (q) and 13.3 (q).

Synthesis of 4-hydroxy-6-pentyl-2-pyrone (**6**):

5-Hexanoyl-4-hydroxy-6-pentyl-2-pyrone (**37**, 12.8 mmoles) was dissolved in 90% H_2SO_4 (10 mL) and heated to 120-130°C for 60 minutes. The reaction mixture was cooled, diluted with 10% $NaHCO_3$ and extracted with ether. The ether extract was dried over anhydrous Na_2SO_4 and concentrated to yield an oil which crystallized under high vacuum. This compound was recrystallized from Skellysolve B:ether:1:1 to yield 1.8 g of the hydroxypyrone **6**. The mother liquors were purified by flash chromatography (30 mm diameter column, 2% MeOH, 2% HOAc in $CHCl_3$) to give in fractions 9 to 13 0.255 g of pyrone **6** (87% yield), mp 52-52.5°C.

TLC: Rf 0.38 in 2% MeOH, 2% HOAc, 96% $CHCl_3$.

FTIR ($CHCl_3$, cast): 2960, 2930, 2845, 2520 (broad), 1690 (w), 1640 (w), 1570, 1540, 1500, 1350, 1310, 1285, 1250, 1140, 920 (broad), 885, 840 and 820 cm^{-1}.

Microanalysis. Calculated: C, 65.92; H, 7.72; O, 26.34. Found: C, 66.04; H, 7.80; O, 26.16.

HREIMS: m/z (formula, intensity, fragment) 182 ($C_{10}H_{14}O_3$, 33, M^+), 164 ($C_{10}H_{12}O_2$, 2, $M-H_2O$), 154 ($C_9H_{14}O_2$, 7, M-CO), 141 ($C_8H_{13}O_2$, 10, $M-C_2HO$), 139 ($C_7H_7O_3$, 11, $M-C_3H_7$), 126 ($C_6H_6O_3$, 100, $M-C_4H_8$), 111 ($C_5H_3O_3$, 72), 98 ($C_5H_6O_2$, 44) and 84 ($C_4H_4O_2$, 64).

^1H NMR (80 MHz, $CDCl_3$): δ 10.9 (1H, bs), 6.1 (1H, s), 5.6 (1H, s), 2.5 (2H, t), 1.7 (2H, bt), 1.4 (4H, m), 0.9 (3H, vt).

3-Hexanoyl-4-hydroxy-6-methyl-2-pyrone (**39**).

Triacetic acid lactone (8 mmoles) was mixed with hexanoyl chloride (11 mmoles) and trifluoroacetic acid (TFA, 5 mL). This solution was heated to reflux for 12 h. The reaction mixture was finally cooled to room temperature and diluted with ethyl acetate before being extracted with distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a white solid which was recrystallized from Skellysolve B to afford 3-hexanoyl-4-hydroxy-6-methyl-2-pyrone (**39**, 67% yield), mp 59-60°C.

TLC: Rf 0.80 in 1% MeOH in $CHCl_3$.

FTIR ($CHCl_3$, cast): 3080, 2940, 2860, 1740, 1720, 1655, 1635, 1615, 1560, 1455, 1350, 1250, 995, 855 and 705 cm^{-1}.

Microanalysis. Calculated: C, 64.27; H, 7.19; O, 28.54. Found: C, 64.14, H, 7.18; O, 28.68.

HREIMS: m/z (formula, intensity, fragment). 224 ($C_{12}H_{16}O_4$, 15, M^+), 206 ($C_{12}H_{14}O_3$, 7, M-H_2O), 181 ($C_9H_9O_4$, 52, M-C_3H_7), 168 ($C_8H_8O_4$, 100, M-C_4H_8), 153 ($C_7H_5O_4$, 79, M-C_5H_{12}).

^{1}H NMR (200 MHz, $CDCl_3$): δ 16.90 (1H, s), 5.98 (1H, s), 3.10 (2H, t, J = 7.5 Hz), 2.30 (3H, s), 1.70 (2H, t, J = 7.5 Hz), 1.40 (4H, m) and 0.93 (3H, vt).

3-Hexanoyl-4-hydroxy-6-methyl-5,6-dihydro-2-pyrone (**40**).

3-Hexanoyl-4-hydroxy-6-methyl-2-pyrone (**39**, 1.33 mmoles) was dissolved in ethyl acetate (20 mL) and mixed with 10% Pd-C (100 mg). This mixture was subjected to a hydrogen pressure of 62 psi in a Parr hydrogenator for 72 h at room temperature. Filtration and in vacuo concentration of the reaction mixture afforded a material which by flash chromatography (30 mm diameter column, 1% MeOH in $CHCl_3$) gave pure 3-hexanoyl-4-hydroxy-6-methyl-5,6-dihydro-2-pyrone (**40**), 170 mg (57% yield), as colorless crystals (mp 38-40°C).

TLC: Rf 0.43 1% MeOH in $CHCl_3$.

FTIR ($CHCl_3$, cast): 2960, 2930, 2850, 1720, 1560, 1460, 1260 and 1060 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ 4.55 (1H, ddq, J = 5, 10, 6 Hz, coupled to δ 2.68, 1.48), 3.05 (2H, 2 x ddd, J = 6.5, 9, 15.5 Hz, coupled to δ 1.70), 1.68 (2H, 2 x dd, J = 5, 17 Hz and 10, 17 Hz, coupled to δ 4.55), 1.70 (2H, m, coupled to δ 3.05, 1.38), 1.48 (3H, d, J = 6.5 Hz, coupled to δ 4.55), 1.38 (4H, m) and 0.92 (3H, vt, J = 7 Hz).

Synthesis of dehydrolachnelluloic acid (**41**).

4-Hydroxy-6-pentyl-2-pyrone (**6**, 1.38 mmoles) was dissolved in TFA (10 mL) and mixed with octanoyl chloride (1.85 mmoles). The reaction mixture was heated to reflux for 24 h. The resulting yellow solution was diluted to 100 mL with ether, and extracted with 10% $NaHCO_3$ (2 x 200 mL), washed with water, dried over anhydrous Na_2SO_4 and concentrated to yield an oily residue. Flash chromatography of this residue (20 mm diameter column, Skellysolve

B:chloroform:2:1) afforded the pure crystalline dehydrolachnelluloic acid (0.4 g, 94%), mp 35-36°C.

TLC: Rf 0.6 in chloroform:Skellysolve B:2:1.

FTIR ($CHCl_3$, cast): 3090 (w), 2960, 2930, 2830, 1725, 1635, 1605, 1560, 1445, 1225, 990, 850 and 700 cm^{-1}.

Microanalysis. Calculated: C, 70.56; H, 8.55; O, 20.89. Found: C, 69.96; H, 9.33; O, 2[illegible]71.

HREIMS: m/z (formula, intensity, fragment) 308 ($C_{18}H_{28}O_4$, 22, M^+), 290 ($C_{18}H_{26}O_3$, 8, M-H_2O), 252 ($C_{14}H_{20}O_4$, 3, M-C_4H_8), 237 ($C_{13}H_{17}O_4$, 64, M-C_5[illegible] ($C_{12}H_{16}O_4$, 100, M-C_6H_{12}), 210 ($C_{11}H_{13}O_4$, 41, M-C_7H_{15}), 204 ($C_{14}H_{21}O$, 31), 168 ($C_8H_8O_4$, 48), 153 ($C_7H_5O_4$,10).

^1H NMR (200 MHz, $CDCl_3$): δ 16.87 (1H, s), 5.92 (1H, s), 3.08 (2H, t, J = 7 Hz), 2.45 (2H, t, J = 8 Hz), 1.67 (4H, m), 1.35 (12H, m), 0.91 (3H, vt) and 0.89 (3H, vt).

Hydrogenation of dehydrolachnelluloic acid (**41**).

Dehydrolachnelluloic acid (0.78 mmoles) was dissolved in ethyl acetate (20 mL) and transferred to a hydrogenation bottle containing 10% Pd-C catalyst (0.05 g). This mixture was pressurized to 60 psi of hydrogen in a Parr hydrogenator for 24 h. TLC of the reaction mixture showed the presence of a product different than lachnelluloic acid. This compound was recrystallized from ether to afford the β-ketolactone **42** in quantitative yield.

TLC: Rf 0.2 chloroform:Skellysolve B:HOAc:2:1:Trace.

FTIR ($CHCl_3$, cast): 2960, 2930, 2865, 1610, 1384, 1250, 1120, 1040 and 740 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ 4.68 (1H, m, coupled to δ 2.73 and δ 2.42), 3.41 (1H, t, J = 7 Hz, coupled with δ 1.92, exchangeable with D_2O), 2.73 (1H, dd, J = 3, 18 Hz, coupled to δ 4.68 and δ 2.42), 2.42 (1H, dd, J = 12.0, 18.0 Hz, coupled to δ 4.68 and δ 2.73), 1.92 (2H, bt, coupled to δ 3.41 and δ 1.3), 1.3 (16H, m) and 0.90 (6H, vt).

Hydrogenation of natural lachnelluloic acid (**1**)

Lachnelluloic acid (**1**, 3.1 mg) was mixed with 5% Pd-C (1 mg) and ethyl acetate (10 mL). The mixture was shaken at 60 psi of hydrogen for 24 h. The reaction mixture was filtered and concentrated to give a white solid which was purified by preparative TLC (20 x 20 cm, 0.35 mm thickness, $CHCl_3$) to afford pure β-ketolactone **42** (3 mg, 100% yield).

TLC: Rf 0.2 $CHCl_3$:Skellysolve B:HOAc:2:1:trace.

^1H NMR (200 MHz, $CDCl_3$): δ 4.70 (1H, m), 3.44 (1H, t), 2.75 (1H, dd), 2.40 (1H, dd), 1.93 (2H, m), 1.80 (2H, m), 1.62 (2H, m), 1.3 (16 to 18 H, bs), 0.93 (3H, vt) and 0.90 (3H, vt).

^{13}C NMR (50 MHz, $CDCl_3$): δ 201.05 (1.7), 169.07 (1.0), 73.81 (3.1), 56.49 (2.0), 43.42 (2.5), 34.23 (2.9), 31.60

(2.3), 31.18 (2.3), 29.44 (2.7), 29.08 (3.6), 29.00 (2.6), 27.02 (2.2), 24.17 (3.0), 22.90 (2.7), 22.40 (3.1), 22.20 (2.6), 13.82 (2.8) and 13.67 (3.4).

HREIMS: m/z 294 ($C_{18}H_{32}O_3$).

Hydrogenation of dehydroacetic acid (**9**)

Dehydroacetic acid (6 mmoles) was mixed with 10% Pd-C catalyst (0.1 g) in a hydrogenation bottle, Ethyl acetate (20 mL) was added to the mixture and the flask was pressurized to 60 psi of hydrogen for 24 h at room temperature. Filtration and concentration of the reaction mixture afforded a yellow solid which was recrystallized from Skellysolve B:acetone to give white crystals of the β-ketolactone **43** in quantitative yield, mp 138-139°C, which gives a negative ferric chloride test.

TLC: Rf 0.3 in Skellysolve B:$CHCl_3$:HOAc:30:70:1.

UV (MeOH): λ_{max} 248 (ε 8130) nm.

FTIR (film): 2960, 2930, 2860, 2540 (broad), 1603, 1385, 1270, 1120, 900 cm^{-1}.

IR ($CHCl_3$): 1760 and 1720 cm^{-1}.

Microanalysis. Calculated: C, 61.52; H, 7.74; O, 30.74. Found: C, 60.12; H, 7.56; O, 32.32.

HREIMS: m/z (formula, intensity, fragment). 156 ($C_8H_{12}O_3$, 8, M^+), 128 ($C_6H_8O_3$, 53, M-C_2H_4), 87 ($C_4H_7O_2$, 9, M-C_4H_5O), 87 ($C_3H_3O_3$, 11, M-C_5H_9) and 55 (C_3H_3O, 100, M-$C_5H_9O_2$).

^{1}H NMR (200 MHz, $CDCl_3$): δ 4.80 (1H, ddq, J = 3, 12, 6 Hz, coupled to δ 2.69, 2.34 and 1.44), 3.32 (1H, t, J = 5.5 Hz, coupled to δ 1.9, slowly exchanged by D_2O), 1.69 (1H, dd, J = 3, 19 Hz, coupled to δ 4.80, 2.34), 2.34 (1H, dd, J = 12, 19 Hz, coupled to δ 4.80, 2.69), 1.90 (2H, m, coupled to δ 3.32, 0.95), 1.44 (3H, d, J = 6 Hz, coupled to δ 4.80), 0.96 (3H, t, J = 7.5 Hz coupled to δ 1.90).

Hydrogenation of triacetic acid lactone (**5**).

Triacetic acid lactone (17.4 mmoles) was mixed with 5% Pd-C (0.2 g) in ethyl acetate (100 mL). The mixture was hydrogenated at 70 psi for 48 h at room temperature. The reaction mixture was filtered and concentrated to give a white solid which was recrystallized from acetone:Skellysolve B to afford pure β-ketolactone **44** in 100% yield, mp 111-113°C ($FeCl_3$ test negative).

TLC: Rf 0.30 1% MeOH, 1% HOAc in $CHCl_3$.

UV (MeOH): λ_{max} 242 (ε 6390) nm.

FTIR (KBr): 3000 (broad), 2700, 1675, 1580, 1380, 1280, 1240, 1050 and 820 cm^{-1}.

Microanalysis. Calculated: C, 56.25; H, 6.29; O, 37.46. Found: C, 56.27; H, 6.41; O, 37.32.

HREIMS: m/z (formula, intensity, fragment) 128 ($C_6H_8O_3$, 38, M^+), 115 ($C_5H_5O_3$, 3, $M-CH_3$), 87 ($C_3H_3O_3$, 2, $M-C_2H_4O$), 84

($C_4H_4O_2$, 4, M-C_2H_4O), 69 (C_4H_5O, 100, M-$C_2H_3O_2$) and 56 (C_3H_4O, 23, M-$C_3H_4O_2$).

^1H NMR (200 MHz, $CDCl_3$): δ 4.78 (1H, ddq, J = 3, 11, 6.5 Hz), 3.55 (1H, d, J = 18 Hz, exchangeable by D_2O), 3.40 (1H, d, J = 18 Hz, exchangeable by D_2O), 2.70 (1H, dd, J = 3, 18 Hz), 2.43 (1H, dd, J = 11, 18 Hz) and 1.50 (3H, d, J = 6.5 Hz).

Hydrogenation of dehydroacetic acid.

This series of experiments were carried out using dehydroacetic acid (**9**, 1.0 g or 6 mmoles), catalyst (0.1 g) and solvent (20 mL). The amount of moderator used was 5 drops or none at all. The reaction mixture was then pressurized in a Parr hydrogenator to the desired pressure. The reaction conditions were varied as shown in Table I . The reaction mixtures were filtratred to eliminate the catalyst and concentrated in vacuo to dryness. The relative ratios of products was determined directly in the crude reaction mixture by ^1H NMR spectroscopy, using characteristic signals for each of the three compounds involved; dehydroacetic acid (**9**) (^1H NMR δ 16.71 (1H, s), 5.92 (1H, bq), 2.68 (3H, s) and 2.27 (3H, bd)), dihydrodehydroacetic acid (**15**) (^1H NMR δ 17.9 (1H, s), 4.65 (1H, ddq), 2.70 (2H, d), 2.68 (3H, s) and 1.50 (3H, d)) and the β-ketolactone **43** (^{1}H NMR δ 4.80 (1H, ddq), 3.32 (1H, t), 2.69 (1H, dd), 2.34 (1H, dd), 1.90 (2H, m), 1.44 (3H, d) and 0.96 (3H, t)).

Hydrogenation of dehydrolachnelluloic acid (**41**) with pyridine as moderator.

Dehydrolachnelluloic acid (5 mg) was mixed with 10% Pd-C (1 mg) in benzene (10 mL) in a hydrogenation bottle. The mixture was pressurized to 60 psi with hydrogen and shaken at room temperature for 6 h. TLC analysis of the reaction mixture showed the presence of starting material (**41**) plus undesired β-ketolactone **42**, but lachnelluloic acid was absent from the reaction mixture. When the hydrogen pressure was lowered to 10 psi the reaction was slower (12 h), but the results were the same.

Hydrogenation of 4-hydroxy-6-pentyl-2-pyrone (**6**).

4-Hydroxy-6-pentyl-2-pyrone (**6**, 1.37 mmoles) was mixed with 5% Pd-C (30 mg) and ethyl acetate (20 mL) in a hydrogenation bottle. The reaction flask was connected to the Parr hydrogenator and pressurized to 70 psi with hydrogen. Under these conditions, the reaction mixture was shaken for 24 h at room temperature. Filtration and concentration of the reaction mixture to dryness afforded 4-oxo-6-pentyl-3,4,5,6-tetrahydro-2-pyrone (**47**) in 100% yield as a white solid (mp 64-65°C).

TLC: Rf 0.33 1% MeOH in $CHCl_3$.

FTIR ($CHCl_3$, cast): 3100, 2960, 2925, 2860, 2570, 1700 (w), 1595, 1390, 1290, 1040, 885 and 830 cm^{-1}.

HREIMS: m/z (formula, intensity, fragment). 184 ($C_{10}H_{16}O_3$, 5, M^+), 166 ($C_{10}H_{14}O_2$, 4, $M-H_2O$), 129 ($C_6H_9O_3$, 24, $M-C_4H_7$), 125 ($C_8H_{13}O$, 29, $M-C_2H_3O_2$), 115 ($C_5H_7O_3$, 17, $M-C_5H_9$), 113 ($C_5H_5O_3$, 100, $M-C_5H_{11}$), 111 ($C_7H_{10}O$, 19, $M-C_4H_9O$).

^1H NMR (400 MHz, $CDCl_3$): δ 4.62 (1H, dddd, J = 3, 5, 8, 12 Hz, coupled to δ 2.70, 2.47, 1.82, 1.71), 3.56 (1H, d, J = 19 Hz, coupled to δ 3.41), 3.41 (1H, d, J = 19 Hz, coupled to δ 3.56), 2.70 (1H, dd, J = 3, 18 Hz, coupled to δ 4.62, 2.47), 2.47 (1H, dd, J = 12, 18 Hz, coupled to δ 4.62, 2.70), 1.82 (1H, dddd, J = 5, 8, 9, 14 Hz, coupled to δ 4.62, 1.71, 1.56, 1.46),1.71 (1H, dddd, J = 5, 5, 11, 18 Hz, coupled to δ 4.62, 1.82, 1.56, 1.46), 1.56 (1H, m), 1.46 (1H, m), 1.36 (4H, m) and 0.92 (3H, vt).

O-Acylation of 4-oxo-6-pentyl-3,4,5,6-tetrahydro-2-pyrone (**47**).

β-Ketolactone **47** (0.71 mmoles) was dissolved in benzene (2 mL) containing DBU (0.73 mmoles) and octanoyl chloride, at 0°C. This mixture was constantly stirred in an ice-water bath for 3 h. The reaction mixture was treated with distilled water (20 mL) and extracted with bezene (2 x 10 mL). The combined benzene extracts were washed with 5% HCl (1 x 20 mL) and brine. The benzene solution was then dried over anhydrous Na_2SO_4 and concentrated to give an oil which was shown to be a single compound (TLC), the enol ester **48** (91% yield).

TLC: Rf 0.8 in Skellysolve B:EtOAc:4:1.

^{1}H NMR (200 MHz, $CDCl_3$): δ 5.82 (1H, s), 4.40 (1H, m), 2.40 (2H, m)

Lachnelluloic Acid (**1**)

6-Pentyl-4-oxyoactanoyl-5,6-dihydro-2-pyrone (**48**, 0.65 mmoles) was dissolved in dry toluene (3 mL) and mixed with DMAP (catalytic amount). The resulting solution was heated to reflux for 5 h. The reaction mixture was finally worked up by adding water and extracting with toluene. The combined toluene extracts were washed with 5% HCl and brine, and after drying over anhydrous Na_2SO_4 and concentrating, an oily residue was obtained. Flash chromatography of this residue (20 mm diameter column, Skellysolve B:EtOAc:95:5:1) afforded, in fraction 7 to 11, pure synthetic lachnelluloic acid (150 mg, 75% yield).

TLC: Rf 0.8 Skellysolve B:EtOAc:HOAc:80:20:1.

FTIR: 2960, 2925, 2860, 1708, 1695, 1560, 1465 and 1070 cm^{-1}.

UV (MeOH): λ_{max} 222 (ε 2685), 274 (ε 5131) nm.

Microanalysis. Calculated: C, 69.64; H, 9.79; O, 20.62. Found: C, 69.72; H, 9.82; O, 20.46.

HREIMS: m/z (formula, intensity, fragment). 310 ($C_{18}H_{30}O_4$, 33, M^+), 292 ($C_{18}H_{28}O_3$, 6.3, M-H_2O), 239 ($C_{13}H_{19}O_9$, 100, M-C_5H_{11}), 226 ($C_{12}H_{18}O_4$, 41, M-C_6H_{12}), 221 ($C_{13}H_{17}O_3$, 55, M-$C_5H_{13}O$), 155 ($C_7H_7O_4$, 17, M-$C_{11}H_{23}$) and 139 ($C_5H_5O_4$, 10, M-$C_{13}H_{25}$).

^1H NMR (200 Mhz, $CDCl_3$): δ 16.8 (1H, s), 4.35 (1H, m), 3.02 (2H, m), 2.61 (2H, 2 x dd), 1.8 (1H, m), 1.63 (3H, m), 1.31 (12H, bm), 0.90 (6H, 2 x vt).

CHAPTER 2 - REFERENCES

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